JOURNAL
OF THE
WASHINGTON ACADEMY OF SCIENCES

VOL. 37 JULY 15, 1947 No. 7

PHYSICS.—*High centrifugal fields.*[1]

J. W. BEAMS, University of Virginia.
(Communicated by KARL F. HERZFELD.)

Gravitational and centrifugal forces no doubt were recognized by man very early in his development. The gravitational pull of the earth and the centrifugal force on his body whenever he moved about in a curved path certainly were ever present. It will be recalled from studies of mechanics that the gravitational force between any two bodies is proportional to the product of their gravitational masses divided by the square of their distance apart, while the centrifugal force is equal to the inertial mass of a moving body multiplied by the square of its velocity divided by the radius of curvature of its path. Also it will be recalled that the gravitational and inertial masses of any given body are proportional. Consequently, the action of a gravitational field and a centrifugal field on matter, for most purposes at least, may be considered to be essentially equivalent (*1*). In view of this equivalence we usually express centrifugal acceleration in terms of the standard acceleration of gravity at the surface of the earth since this is one of our most familiar concepts.

The intimate contact with centrifugal force in our every-day life undoubtedly accounts for its wide use in a multitude of our activities. One of its important uses, which we shall now attempt to discuss, is the production and application of high centrifugal fields to a few problems in physics, chemistry, biology, and medicine. Space does not permit a complete review of this rapidly growing use of centrifugal fields, so a large amount of important work necessarily will have to be passed over. Emphasis will be placed upon certain problems not because they are thought to be more important than others but rather because my colleagues and I happen to have had more firsthand experience with them in our laboratory at the University of Virginia.

One important use of centrifugal fields is in the purification or separation of different substances and in the determination of particle or molecular weights, sizes, and shapes. It is, of course, common knowledge that larger particles settle out of a liquid or a gas faster than smaller ones. Just one hundred years ago Stokes (*2*) showed that the velocity of fall v of a spherical particle of density ρ_p and radius a in a liquid of density ρ_d and coefficient of viscosity η, in a gravitational or centrifugal field F, is given by the relation

$$v = \frac{2}{9\eta}\left(\rho_p - \rho_d\right) a^2 F. \qquad (1)$$

It will be observed from this relation that the rate of settling in either a gravitational or centrifugal field increases with density and with size of particle and hence may be used for separating particles of different sizes and different densities. However, from the above equation of Stokes alone, all uncharged particles or molecules in a convection-free suspension or solution might be expected to settle out after a sufficient time has elapsed. This, of course, is contrary to observation since, for example, an unsaturated solution of sugar or salt may be left to stand or centrifuged in a low-speed centrifuge as long as desired without appreciable sedimentation. This apparent absence of sedimentation is due to the fact that diffusion always opposes sedimentation. Diffusion arises from the thermal agi-

[1] The sixteenth Joseph Henry Lecture of the Philosophical Society of Washington, delivered at the meeting of the Society on February 15, 1947. Received March 5, 1947.

tation or Brownian motion of the particles and molecules and tends to produce a transport of the particles or molecules from regions of high concentration to regions of low concentration. If the particles are large enough, their velocity of sedimentation in the gravitational field or low-speed centrifuge is much larger than their back diffusion, and so they are precipitated. However, as the size of the particle decreases the magnitude of the centrifugal force must correspondingly be increased in order to overcome the increased back diffusion. When the particle reaches molecular dimensions the centrifugal field required for appreciable sedimentation becomes quite large, but with the centrifuging technique now available appreciable sedimentation of almost any molecules or atoms can be obtained.

Unfortunately, the general theory of sedimentation in real liquids has never been worked out completely, but a good theory does exist for sedimentation in ideal incompressible liquids. Mason and Weaver (*3*) solved this problem for sedimentation in a uniform gravitational field in 1924, and Archibald solved it for sedimentation in a centrifuge in 1938 (*4*). According to Lamm (*5*) the differential equation for sedimentation of uncharged particles or molecules in an ideal, dilute, incompressible solution enclosed in a sector shaped centrifuge cell rotating with an angular velocity ω is given by

$$\frac{1}{r}\frac{\partial}{\partial r}\left\{\left(D\frac{\partial c}{\partial r} - \omega^2 rSc\right)r = \frac{\partial c}{\partial t}, \quad (2)$$

where r is the distance of the material from the axis of rotation, c is the concentration, t the time, D the diffusion constant, and S the Svedberg sedimentation constant or the velocity of settling of the solute or particles in unit centrifugal field. Faxen (*6*) and Soyten (*7*) each have given approximate solutions to this equation, and Archibald has given the general solution. Recently Archibald has greatly simplified the calculations necessary in applying the theory to experimental results (*8*). Many years ago Svedberg (*9*) derived the equations for sedimentation in a sector-shaped centrifuge cell (equation 2 above) for two most important special cases. In the first case he assumed that if the centrifuging process is continued long enough, diffusion will exactly balance the sedimentation and equilibrium will be established, i.e., $\partial c/\partial t = 0$, and the change in free energy throughout the centrifuge vanishes. This gives

$$M_e = \frac{2\,RT\,ln\,c_1/c_2}{(1 - V\rho)\omega^2(r_1^2 - r_2^2)} \quad (3)$$

and

$$V_e = \frac{2\,RT\,ln\,c_1/c_2}{N(\rho_p - \rho_d)\omega^2(r_1^2 - r_2^2)}, \quad (4)$$

where M_e is the particle or molecular weight, V_e is the volume of the particle or molecule, c_1 and c_2 are the concentrations at distances r_1 and r_2 from the axis of rotation, respectively, ρ, ρ_p, and ρ_d are the densities of the solution, particle and solvent respectively, V is the partial specific volume of the substance, R is the gas constant, N is the Avogadro number, and T is the absolute temperature. It will be observed that equation (3) is merely the statement of the Maxwell-Boltzmann distribution in the centrifugal field. It subsequently has been derived from thermodynamic reasoning by Svedberg and Tiselius. If the solution is an electrolyte which dissociates into m n-valent cations and n m-valent anions, where the numbers n and m contain no whole number factor,

$$M_e = \frac{2(m + n)\,RT\,ln\,c_1 f_1^{\pm}/c_2 f_2^{\pm}}{(1 - V\rho)\omega^2\,(r_1^2 - r_2^2)}, \quad (5)$$

where $f_1^{\pm}$ and $f_2^{\pm}$ are the activity coefficients (*10*). In most cases where the molecular weight is comparatively large and the solutions are not at the isoelectric point, or where the particles are charged, the electrostatic effects of the ions can be repressed by the addition of a low molecular weight electrolyte such as NaCl and the simpler equations (3) and (4) may be used instead of (5). Electrical charges on the sedimenting particles always reduce the velocity of sedimentation. In case the solute is ionized the sedimentation of the heavier ion is decreased and that of the lighter ion is in-

creased since the heavier ion drags the lighter ion along with it. When the centrifugal field is first applied to a solution containing ions an electrical potential appears between the axis and periphery. This electrical potential decreases as the sedimentation continues and vanishes when equilibrium occurs (*11*, *12*).

In applying the above equations the concentrations and temperature are measured under equilibrium conditions, and hence to a first approximation the values of M_s are independent of particle or molecular shape. The partial specific volume V is usually measured outside the centrifuge and does not take account of hydration or solvation effects. However, Lamm (*10*), Kraemer (*10*), Pickels (*13*), and their collaborators have shown how to correct for these effects and have found that, in most practical cases, the correction is comparatively small, although there are exceptions. In cases where the centrifuge cell contains a solution with a number of different solutes equation (3) gives the concentration of each substance independently of the others as a function of the radius provided the solution is sufficiently dilute. Consequently by measuring the concentration in the centrifuge cell at a number of different radii, it is possible to determine the distribution of particle weights in the solution.

Although the above equilibrium method of equations (3), (4), and (5) gives most reliable values for M_s and V_s, the centrifuging time required for equilibrium to be established is very long when the molecular weight M_s is large. In fact, for many of the biologically important substances which have large molecular weights, this equilibrium time is of the order of several days or weeks.

In order to avoid this long centrifuging time Svedberg (*9*, *20*) devised the rate of sedimentation method which is especially applicable to the measurement of the molecular weights and sizes of large molecular weight substances. In this rate of sedimentation method both the molecular weight and centrifugal field must be large enough to produce a sedimentation velocity that can be measured directly. If the particles or molecules are uncharged and the solution is ideal and dilute and if the experimental conditions are such that molecular or particle reflection from the ends of the sedimenting column can be neglected, then the sedimentation force per mole may be equated to the frictional force per mole

$$M_s(1 - V\rho)\omega^2 r = f\frac{dr}{dt}. \qquad (6)$$

The frictional force $f = RT/D$ where D is the diffusion constant. This gives for the molecular weight M_s

$$M_s = \frac{RT}{D(1 - V\rho)}\frac{dr/dt}{\omega^2 r} = \frac{RT}{D(1 - \rho V)}S, \qquad (7)$$

where S is the velocity of sedimentation in a unit field, and is usually expressed in Svedberg units, i.e., in units of centimeters per second per unit field of force multiplied by 10^{13}. Actually most of the values given for S have been reduced to the equivalent velocity of sedimentation S_{20} in water at 20°C by the relation

$$\frac{S_t}{S_{20}} = \frac{\eta_{20}}{\eta_t}\frac{1 - V_t\rho_t}{1 - V_{20}\rho_{20}}.$$

In this method each molecular species gives rise to an easily distinguishable boundary between the solvent and the sedimenting column so that dr/dt and hence S can be quite accurately measured. As the time of centrifuging increases, this sedimenting boundary becomes progressively blurred because of diffusion. However, for a comparatively large molecular weight substance, this blurring is small, and furthermore diffusion theory shows that the position which the boundary would have occupied had there been no diffusion is the place where the concentration is one half that in the unaffected sedimenting column adjacent to the boundary. The concentration in the column of sedimenting material decreases slowly as the centrifuging progresses since the particles move out along the radius and hence diverge. Also the centrifugal field increases toward the periphery which

in turn increases the rate of sedimentation and hence reduces the concentration in the sedimenting column. If c_o is the original concentration and c_t is the concentration at any time, t, then Svedberg and Rinde (*9*, *10*) have shown that

$$c_t = c_o \frac{r_o^2}{r_t^2} \tag{8}$$

where r_o is the radius of the meniscus and r_t is the radius of the boundary at the time t. When the solution contains several sedimenting substances, each forms its characteristic sedimenting boundary so that a molecular weight analysis of the solution is automatically obtained. Also if the solution contains a distribution of molecular weights and sizes rather than a homogeneous molecular species, the rate of sedimentation method can give this distribution.

It will be observed from equation (7) that in order to get the molecular weight M, the diffusion coefficient D must be measured. This may be carried out either during the centrifuging process or in separate experiments. Both methods give essentially the same results so the latter method is often used. If the particles or molecules are known to be spherical and hydration effects can be neglected, the frictional constant f_o can be calculated from the following equation of Stokes:

$$f_o = 6\pi\eta N\left(\frac{3MV}{4\pi N}\right)^{1/3}, \tag{9}$$

which when substituted into equation (6) gives the molecular weight M. The ratio of the measured frictional constant f of equation (6) to the calculated value f_o [equation (9)] is always equal to or greater than one. If the amount of hydration is known the approximate shape of the molecules or particles can be estimated.

The equilibrium method [equation (3)], which has its greatest application in the smaller molecular weight range, and the rate of sedimentation method [equation (7)], which is adapted principally to the larger molecular weight range, recently have been supplemented by a method made possible by the solution of equation (2) by Archibald. Archibald (*8*) has put the results of his mathematical solution in a convenient form for use, and it is hoped that it will provide increased reliability for the results especially in the large molecular weight range.

Although the above outline of the theory is very brief and incomplete, it does show that a sufficiently powerful centrifuge may be used as an analytical tool for the determination of particle or molecular weights, sizes, and shapes or that it may be utilized for the purification of materials or the separation of a mixture into its various pure components. The value of this tool in biological, and medical research is enhanced by the fortunate fact that most of the biological compounds in solution are not deactivated by high centrifugal fields so that the measurements obtained are for this material in its natural state.

For analytical work the centrifuge must produce a centrifugal field sufficiently intense to give an appreciable sedimentation and the solution being centrifuged must be convection free. It can be shown that in order to meet the latter requirement the centrifuge cell should be sector shaped so that the sedimenting particles can all move freely in the direction of the centrifugal field and that the centrifuge cell should be at as nearly uniform temperature as possible.

Svedberg Ultracentrifuges

Svedberg and his associates not only pioneered in the use of the convection free centrifuge for analytical work, but they have provided us with a major portion of the molecular weight data obtained so far by the centrifuge method (*10*). No attempt will be made here to discuss the Svedberg ultracentrifuges, since they are described in detail in a book by Svedberg and Pederson (*10*), except to mention that they consist of two principal types. The first type develops comparatively low centrifugal fields (500 to 15,000 g). It is driven by an electrical motor and supported in ball bearings. The rotor spins in hydrogen and carries a sector-shaped cell with quartz windows which contains the material to be centrifuged. The sedimentation is viewed optically through the quartz windows. This type of ultracentrifuge is used principally

for sedimentation equilibrium measurements. The second type of ultracentrifuge develops much higher centrifugal fields (15,000 to 750,000 g) and is driven by twin oil turbines. The material being centrifuged also is contained in a sector-shaped cell, which has quartz windows so that the sedimentation can be recorded by optical methods. The rotor is surrounded by hydrogen at about 20 mm pressure which conducts the heat generated by the friction in the bearings, by gaseous friction on the rotor, and by oil impinging on the turbines, to the casing. This type of ultracentrifuge has been used primarily for rate of sedimentation measurements.

AIR-DRIVEN VACUUM-TYPE ULTRACENTRIFUGE

The vacuum-type air-driven ultracentrifuge originally was developed to avoid the troublesome effects of air or gaseous friction on high-speed rotors or centrifuges (*14*, *15*, *16*) This gaseous friction at high rotor speeds not only consumes a large amount of power but also it heats the centrifuge rotor. This heating of the centrifuge rotor is greater on the periphery than on the axis, and thermal gradients in the rotor are set up which cause convection in the centrifuge cell. Since the forces that produce this convection increase with the centrifugal field thermal gradients necessarily should be as small as possible if the data are to be reliable. Essentially this machine consists of a large centrifuge rotor located inside a vacuum-tight chamber, a small air-driven air-supported turbine situated above or below the chamber, and a thin flexible shaft which fastens them together and which is coaxial with their common axis of rotation. Several variations in the design of these convection-free vacuum-type ultracentrifuges are in use by different workers (*16*), but Fig. 1 gives a diagram of a machine used at Virginia for analytical work which will serve to illustrate the principles of the apparatus (*17*). The rotating members consist of the flexible steel shafts A (0.1 inch) and A'(0.1 inch), the driving turbine T, the centrifuge rotor RA, and a magnet M. The shafts are vertical and coaxial and turn in the hard babbitt bearings G_1, G_2, G_3 and a very loose (15-mil clearance) sliding guide SB. G_1, G_2, and G_3 are mounted in flexible round neoprene rings and are oil sealed. G_3 is lubricated and sealed with a low vapor pressure oil so that a good vacuum can be maintained in the steel vacuum chamber V. The guide bearing SB serves to prevent "swinging" of the rotor at low rotational speed. At running speed the shaft A' runs free without touching in the 15-mil clearance. The rotating members are supported by an air-thrust bearing formed between the Bakelite collar B and the turbine T. This collar is fastened to a flexible neoprene support D. The centrifuge rotor RA is a Duralumin analytical rotor with the cell C, which contains the solution to be centrifuged 65 mm from the axis of rotation. Fig. 2 shows a picture of this rotor and the cell. The rotor is made oval shaped to increase its strength, and the cell is sector shaped with crystal quartz windows cut perpendicular to the optic axis for observing the sedimentation optically. The permanent magnet M together with the field FC provides a speed control for the ultracentrifuge. M is a magnetized, hardened steel rectangular bar $1\frac{1}{2}$ inches long, $\frac{1}{4}$ inch wide, and 9/16 inch high. It has a hole in the center for the shaft A, to which it is rigidly attached by set screws or a steel clutch. The field coils FC consist of a large number of turns of well-insulated copper wire wound on a laminated field core. The laminations were (0.02 to 0.0075 inch thick) silicon steel laminations to give a total thickness of 9/16 inch. The copper wire was no. 21 enameled, and sufficient turns were used to give an inductance greater than 30 millihenrys for the coils connected in series.

To operate the centrifuge the vacuum chamber V and rotor RA are first thermostated to the desired running temperature. The material to be centrifuged is then placed in the vacuum-tight centrifuge cell which is tightly sealed and placed in RA. Electrical condensers are next connected in series with the field coils FC, which possess the proper capacity to make the circuit resonate at a frequency slightly above the desired running speed. Oil is next forced into G_1, G_2, and G_3 and the vacuum chamber V evacuated to less than 10^{-5} mm Hg through

P. Compressed air then is applied through *SI* to the air support at a pressure (10 to 15 lbs/in^2), which allows the rotating members to turn freely. The driving air is next admitted through *DI* to the turbine *T*, and the centrifuge starts spinning. The centrifuge will continue to accelerate until electrical resonance starts in the circuit. The rotating magnet *M* induces an e.m.f. in the field coils with a frequency equal to its frequency of rotation, but the current in the circuit is negligible until the circuit starts resonating. When resonance starts the current in the circuit increases abruptly and the reaction is such as to brake the magnet. If now the air pressure to the turbine is set a few lbs/in^2 above that necessary to maintain this speed of the centrifuge, the resonant circuit will absorb the surplus energy, and the rotor speed will be maintained constant. In practice, with an ordinary air-pressure regulator in the air line, the speed of the centrifuge automatically remains constant to less than 0.1 per cent as long as desired. Also, if no changes are made in the driving air pressure or circuit, the rotor speeds will check to about 0.1 per cent on successive runs. An idea of the critical braking action of this speed control can be obtained from the fact that, in our experiments, doubling the driving air pressure increased the rotor speed (at about 1,000 r.p.s.) by less than 1.5 per cent. Also our air turbines are not able to drive the centrifuge through resonance with six times the normal driving air pressure, which was all we had available on our pressure line. It will be observed that no over-heating occurs when energy is absorbed in the control due to the fanning action of the magnet *M*.

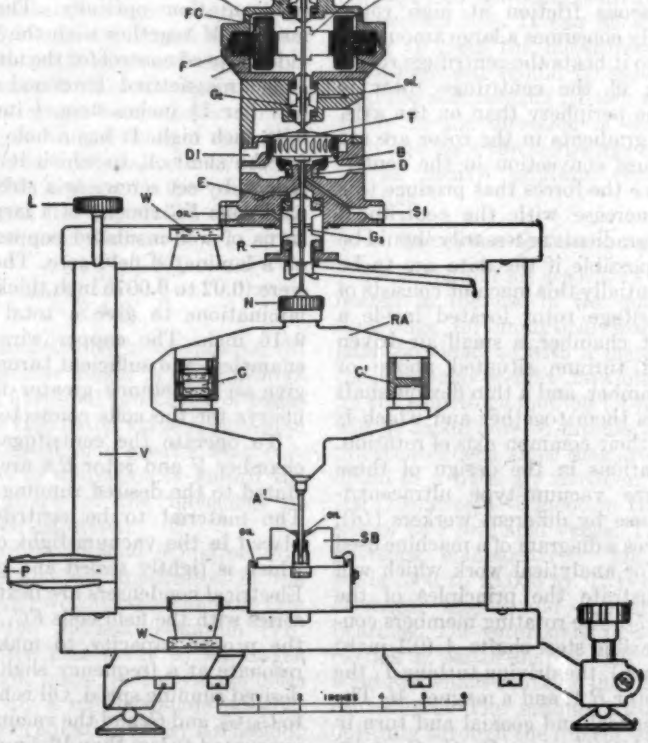


FIG. 1.—Air-driven air-supported vacuum-type ultracentrifuge with speed control.

Also any heat from the control is isolated from the vacuum chamber by the air passing through the air turbine below it. It should be pointed out that the only factor that limits the maximum centrifuge rotor speed is the mechanical strength of the rotor. Clearly the running speed can be set anywhere below this value. Since the centrifuge rotor spins in a good vacuum, no appreciable heat is developed and hence no temperature gradients are formed in the centrifuge cell. In some experiments hydrogen at a pressure of a few mm of Hg has been used to surround the rotor. In these experiments it was desirable to have a good thermal connection between the vacuum chamber walls V and the rotor. The low viscosity and hence low resultant rotor friction of hydrogen together with its comparatively large heat conductivity make it the best gas to use for this purpose. If the pressure of the hydrogen in V is not over a few mm Hg, convection-free sedimentation is obtained.

Several methods are available for measuring the concentration or sedimentation in the centrifuge cell optically. Most of these methods make use of the fact that the solute has a different absorption spectrum than the solvent or they employ the gradient of the refractive index in the cell. For many proteins, in order to get sufficient differential absorption, ultraviolet light must be used. This requires quartz optical parts throughout and hence is rather expensive. However, for the refractive index gradient methods, glass is used. Except in certain special cases the refractive index methods are preferable and a number of modifications of it are in use. Lamm (*18*, *10*) developed a method in which a scale is photographed through the centrifuge cell. From the distortions in the images of this scale the refractive index gradient is determined. Philpot (*19*), Svensson (*20*), Anderson (*21*), Pickels (*13*), Williams (*22*), and others have used methods based upon the Toepler schlieren idea. Special tests carried out by Pickels showed that deviations of the light beam produced at any level of the centrifuge cell (at least up to 0.02 radian) can be determined photographically with a precision of less than 1 per cent and that the image positions of every radial distance in the cell can be produced with an accuracy of less than 1 per cent of the total centrifuge cell height. In our laboratory at Virginia, I. Foster is using a Jamin interferometer method for measuring the sedimentation in very dilute solutions. It is obvious, of course, that other than optical methods may be used for measuring sedimentation such as the radioactive radiations from atoms tagged to the sedimenting molecules, etc. It will be recalled that the present theory of sedimentation holds only for dilute solutions, therefore it is important in some cases at least to use a method of recording the sedimentation in as dilute solutions as possible. There is indeed a genuine need for better methods of recording sedimentation in very dilute solutions than are in use at present.

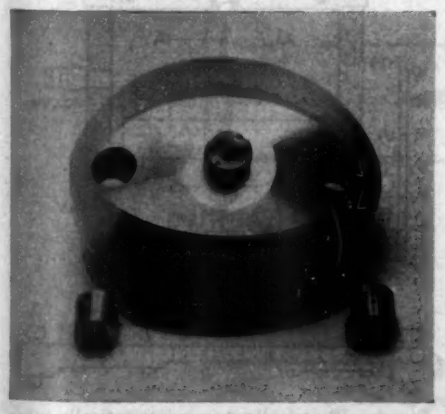


FIG. 2.—Analytical ultracentrifuge rotor.

ELECTRICALLY DRIVEN MAGNETICALLY SUPPORTED VACUUM-TYPE ULTRACENTRIFUGE

The air-driven air-supported vacuum-type ultracentrifuge just described possesses practically all of the desirable features of an ideal centrifuge. However, it requires a supply of compressed air, which is not always readily available. Also a noisy air compressor is sometimes a nuisance to be avoided. Because of this we (*23*, *24*, *16*) developed at Virginia before the war the electrically driven magnetically

supported vacuum-type ultracentrifuge shown in Fig. 3. The rotating components consist of the steel motor armature *D*, the steel magnetic support core *R*, the flexible shaft *A*, and the centrifuge rotor *C*. The shaft is hollow from G_3 to G_2, through which cooling water passes. The rotor *C*, flexible shaft *A*, vacuum chamber *V*, and oil glands G_1 and G_2 are the same as in the air-driven vacuum-type centrifuge described previously. The only differences are that an electrical motor is substituted for the air drive and a magnetic support is used instead of an air support. The small remaining weight is carried by a small thrust bearing consisting of the upper babbitt surface of G_3 and a small collar on the shaft. This small oil thrust bearing is designed to support the total weight of the rotating system in case the power supply of the lifting solenoid should fail. This type of magnetic support is almost friction free. The four field coils *F* of the motor are supplied with a high-frequency 2-phase alternating current in such a way that the field rotates with the same frequency as the alternating current. This rotating field induces currents in the steel armature *D* and causes it to rotate. This type of induction motor is well suited to this problem because a comparatively large starting and accelerating torque exists. Several ways may be used to produce the alternating power required. At Virginia an electronic generator is used with which the rotational speed of the centrifuge remains constant to 0.1 per cent as long as one desires. Fig. 4 shows a couple of rotor speed versus time curves taken on successive days. The entire apparatus operates from the 110-volt AC lines and is automatic. With the 6-inch Duralumin rotor of Fig. 3 weighing about 9 pounds, 1 kilowatt input to the motor at a frequency of 1,180 cycles per second accelerated the rotor to 1,000 r.p.s. in a little over 15 minutes, which compares favorably with the air drive. The power input to the motor required to maintain the speed constant at 1,000 r.p.s. was less than 500 watts.

The precision with which molecular weights can be measured with the air-drive (Fig. 1) and electrically driven (Fig. 3) centrifuges is about the same. In most experiments the rotational speed can be measured to 0.1 per cent, the temperature to about 0.1°C, and the rate of sedimentation to at least 1 per cent. In the equilibrium method the ratio of the concentrations at the various radial distances of the cell can be measured to less than 1 per cent and the temperature and rotor speed held constant for the run to at least 0.1 per cent. The most uncertain element in the molecular weight determination of ultracentrifugation is in finding the true value for the partial specific volume *V*. As pointed out before this arises from the uncertain knowledge as to the amount of hydration or solvation present. There is urgent need for a reliable method of determining *V* in the actual solution. The three centrifuge methods, including the equilibrium, the rate of sedimentation, and the new method which makes use of Archibald's solution of equation 2, provide comparatively good molecular-weight data over a range in

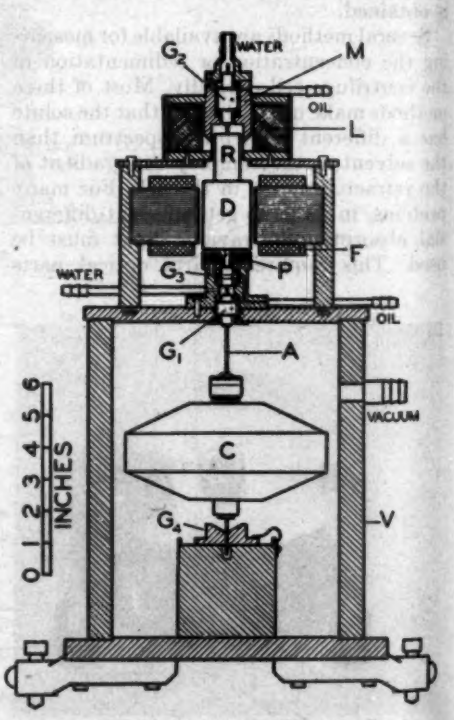


FIG. 3.—Electrically driven magnetically supported vacuum-type ultracentrifuge.

molecular weights from the lightest to the heaviest molecules known. The first equilibrium method (equations 3, 4, and 5) is used primarily for molecular weights below about 10,000, while the latter two methods are best suited for molecular weights above 10,000.

From the standpoint of biophysics, one of the most interesting facts that have come out of the molecular-weight data is the apparent existence in nature of very high molecular weight homogeneous compounds. Some of these compounds have molecular weights that exceed 10^7 molecular-weight units. These compounds are quite stable and, in some cases as far as can be determined, are composed of a single molecular species in which each molecule has exactly the same mass. Not all of these naturally occurring substances such as keratin, fibroin, and myosin are composed of homogeneous molecular species, but most of the easily soluble proteins have definite molecular weights. In some cases such as the respiratory proteins, which have been extensively studied in the analytical ultracentrifuge, the molecules dissociate into submultiples. For example, when a solution of haemoglobin is made very dilute, the molecules split into halves. In the case of a haemocyanin (*Helix pomatia*) solution at the isoelectric point, whole molecules exist alone with molecular weight of 8.9×10^6 provided the salt concentration is not too high. When the solution is made weakly acid, half molecules appear. If it is made alkaline, half molecules appear first followed by molecules one-eighth the original molecular weight, etc. Within the region of *p*H 4 to *p*H 9 the dissociation is reversible and the whole molecules will be reformed at the isoelectric point. In the case of some proteins Svedberg and, more recently, Pedersen (*10*) have interpreted their molecular-weight data as indicating that certain proteins they have studied have molecular weights that are integral multiples of 17,600. In other words, the building blocks of these proteins have a mass of 17,600 molecular-weight units. These conclusions have been questioned by a number of workers (*25*, *26*) on the ground that the molecular-weight data are not good enough definitely to prove that the 17,600 unit exists. Whatever may be the answer to this most interesting and important suggestion of Svedberg, all workers in the field will agree that it would be highly desirable to obtain more precise data if it is possible. For example, an error of ± 1 per cent in a molecular weight of 10^7 is $\pm 10^5$ molecular units. Just before the war some experiments (*27*) were started at Virginia with the hope of examining more closely the homogeneity of some of these proteins, but these have been interrupted until the past few months.

Several alternate methods were considered including the electron microscope. This beautiful method, while giving the shape of the largest molecules, has its precision

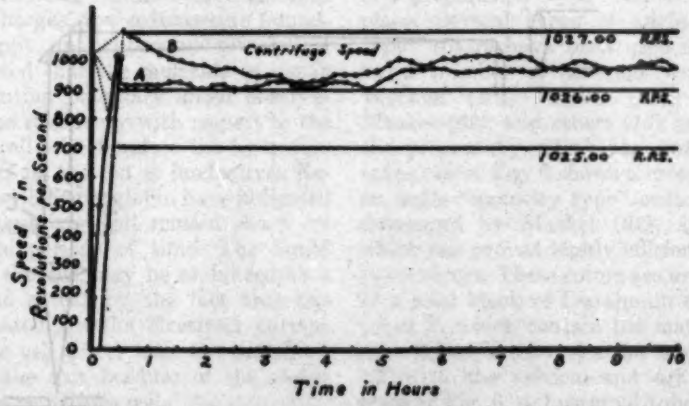


FIG. 4.—Rotor speed versus time curves on successive days for the electrically driven magnetically supported ultracentrifuge.

limited by a resolving power of from 10 to 20 Ångstroms (*28*). It also may deactivate the molecules under study. Therefore it seemed necessary to reexamine the centrifuge method, especially since the electron microscope technique was being vigorously pursued elsewhere.

In the rate of sedimentation method the resolving power or ability of the centrifuge to resolve two molecular species is approximately proportional to $\omega^2 rh$ where $\omega^2 r$ is the centrifugal field and h is the height of the cell. The strength of a centrifuge rotor is roughly proportional to $\omega^2 r^2$ where ω is the angular velocity and r is the radius of the rotor. In practice $\omega^2 rh$ is very roughly proportional to $\omega^2 r^2$, and so the resolving power is proportional to the bursting strength of the centrifuge rotor. Therefore, since, in the present centrifuges, the rotor speed is limited only by the strength of the rotor, there does not seem to be much promise of increasing the resolving power by increasing the rotor strength at least until better rotor material is available. However, if we let T be the time that the sedimenting boundary remains in the field of view of the centrifuge cell, the resolving power becomes $(\omega^2 r)^2 ST$ or directly proportional to T. Now it is possible to increase the time T by flowing the solvent through the centrifuge cell at approximately the same speed with which the material settles out. This makes it possible to keep the sedimenting boundary in the field of view for long periods of time, thus greatly increasing the resolving power of the machine. Fig. 5 shows a schematic diagram of the arrangement in which the straight schlieren method is used for observing the sedimenting boundary. The solvent is injected at a controllable rate through the axis of the spinning centrifuge and flows through the centrifuge cell from the periphery toward the axis where it is collected in a large cavity in the rotor. If there is any nonhomogeneity in the substance being centrifuged, or if it consists of two or more

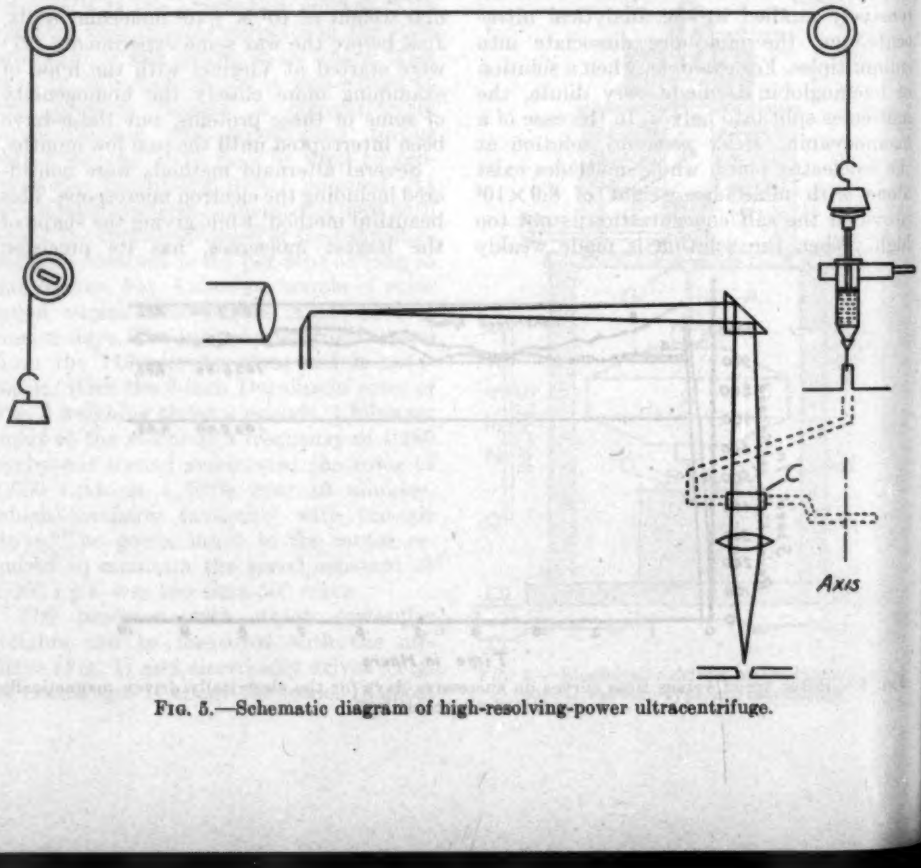


FIG. 5.—Schematic diagram of high-resolving-power ultracentrifuge.

molecular species, the sedimenting boundary will blur or split up into two or more sedimenting boundaries respectively. In some preliminary experiments haemoglobin was apparently homogeneous to at least 50 molecular-weight units. The ultimate limiting factor with the method seems to be a gradual dilution produced by the effect described in equation (8), but, with better optical methods of measuring sedimentation in dilute solutions, the method has promise of precision much greater than any yet obtained.

A second method of testing the homogeneity of large molecular weight substances also is being developed at Virginia. In this method the sedimentation in the cell is balanced by electrophoresis. Electrodes are placed in the centrifuge cell in such a way that a current can be passed through the cell along the radius. The solution containing the materials to be centrifuged is brought to a *p*H value not on the isoelectric point. The centrifuge is then spun to full speed and sedimentation allowed to take place until the sedimenting boundary moves to the middle of the cell. The electrical current is then passed through the cell in such a direction that this boundary remains stationary. Under these conditions, if the solute that forms the sedimenting boundary is either nonhomogeneous or composed of different molecular species, the sedimenting boundary will no longer remain sharp. Also, if the molecules or ions in the sedimenting columns have different electrical charges, the sedimenting boundary will split up or become blurred. It may be noted that the molecule or ion in the sedimenting boundary under observation remains stationary with respect to the centrifuge cell and therefore the hydration or solvation of the ion is ineffective. Experiments with haemoglobin have indicated that the boundary will remain sharp for considerable periods of time. The liquid column in the cell may be stabilized to a considerable extent by the fact that the heat generated by the electrical current through the cell is less near the periphery than near the axis because of the sector shape of the centrifuge cell.

The high-speed centrifuge not only may be employed as an analytical tool as discussed above but also it may be used as a means of purifying or separating substances that have different rates of sedimentation or different densities. As a matter of fact, this latter function is perhaps more important than the former. A great many substances of utmost importance in biology and medicine exist in nature in very dilute solution and in mixtures. In order properly to study these substances, it is usually necessary to purify and concentrate them by some means which does not destroy their biological activity. Often comparatively small changes in *p*H or temperature will destroy them so that the usual chemical means of concentration cannot be used. However, as mentioned previously, a high centrifugal field does not seem to deactivate them in any way; also the temperature and *p*H in the vacuum type ultracentrifuge can be accurately adjusted to any desired value within the working range. In most cases it is desirable to centrifuge comparatively large amounts of the natural solution in order to obtain a sufficient quantity of the purified substance to carry out the desired experiments.

The vacuum-type high-speed ultracentrifuges described in Figs. 1 and 3 may be used for the purification or concentration of substances instead of as an analytical centrifuge by simply removing the analytical centrifuge rotor from the shaft and placing a so-called angle "quantity type" or "preparation type" centrifuge in its place. Several types of angle "quantity type" rotors have been used successfully by a number of different workers, and Wyckoff (*30*), Pickels (*31*), Rosenfeld, Masket (*32*), and others (*16*) have studied the process by which the sedimentation takes place. Fig. 6 shows a cross section of an angle "quantity type" centrifuge rotor developed by Masket (*32*), at Virginia, which has proved highly efficient and easy to construct. These rotors are usually made of a solid block of Duralumin ST 14. The tubes *T*, which contain the material to be centrifuged, are bored at an angle of about 12° with the vertical and are drawn to scale in Fig. 6. A Lusteroid tube which fits snugly into *T* is completely filled with the

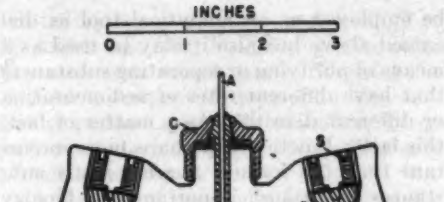


FIG. 6.—Preparative or "quantity" type ultracentrifuge rotor.

solution or suspension to be centrifuged. A leak-tight plug P is forced into T and the screw S tightened until a leak-tight seal is made. The tubes T are accurately spaced so that the rotor is in good dynamical balance when either full or empty. The rotor shown in Fig. 6 holds about 100 cc when all the tubes are filled, and the maximum centrifugal field in the cell is about 300,000 gravity. With the apparatus of Figs. 1 and 3, the strength of the rotor Fig. 6 is the only factor that limits the rotor speed and centrifugal field. The strength of a homogeneous centrifuge rotor depends not on its absolute strength directly, but upon this divided by the density of the rotor material. This is not quite true for the rotor of Fig. 6, which carried 100 cc of solution, but it is roughly correct. Duralumin ST 14, therefore, is almost as good as steel and possesses the added advantage of being machinable after heat treatment. Also it is considerably lighter, which allows it to be accelerated rapidly and lessens the damage done if an accidental explosion occurs. On the other hand, for the substances of lower molecular weights, high strength steel no doubt could be used to a considerable advantage. The sedimentation that takes place in the tubes T is not completely convection free as in the case of the analytical rotors, but what convection exists aids the separation instead of hindering it. The material settles out almost to the outer wall and thus increases the density in this outer region. The force on this concentrated solution is then such as to cause is to move down to the bottom of the tube where it settles out. Several of the viruses actually crystallize out in the bottom of the tube. After the sedimentation is completed, the centrifuge rotor is allowed to decelerate to zero. The rotor may be decelerated rather rapidly down to about 300 r.p.s. and then move slowly on down

FIG. 7.—Schematic diagram of tubular centrifuge rotor.

to rest. The deceleration must be smooth in order to avoid remixing. The Lusteroid tubes which contain the material to be centrifuged are carefully removed from the rotor and placed in a vertical holder. The lighter fractions are then drawn off through a hypodermic needle or other arrangement which does not stir or remix the contents of the tube. Sometimes, when no material is actually deposited on the bottom of the tube, a method of displacement with a heavier nonmixable liquid may be used to separate the lighter from the heavier fractions. Pickels (*31*), Masket (*32*), and others have shown that a fairly precise analysis of the molecular weights of the substances being centrifuged can be obtained if this procedure is carried out with care. This analytical technique is most useful in many cases where the substance being concentrated is a very dilute active agent whose absorption spectrum or other optical properties are unknown.

In many cases it is desirable to process a very large volume of material, and then the above "quantity type" of high-speed centrifuge becomes impracticable. About 10 years ago at Virginia we attacked this problem by means of a continuous-flow tubular high-speed centrifuge (*16*). This work was dropped in 1940 because of the war and has not as yet been taken up again, although the results showed that the method was strikingly successful.

Fig. 7 shows a schematic diagram of the tubular centrifuge rotor used. This rotor consists of two concentric steel cylinders with radii R_1 and R_2 and length L. The inner cylinder is solid steel. The material to be centrifuged enters at I and then flows vertically down the annular space between the two cylinders where sedimentation takes place. The light fraction emerges through L and the heavy fraction through H. If the inner wall of the outer cylinder is accurately cylindrical some of the heavy material settles out on the wall, but if it is slightly cone shaped with the apex of the cone toward the top, the concentrated material will move down along the wall and if the exits are properly made will emerge through H, provided of course the concentration is not allowed to become so great that it clogs the outlets. However, in most practical cases, the sedimented material is in dilute solution and it is desirable to have the concentrated material deposited on the wall. In practice, H is plugged and this material is deposited in a smooth film or coating on the inside of the outer cylinder and does not interfere with the sedimentation until it becomes too thick. At this stage the centrifuge rotor is stopped and the film may be ripped or scraped off of the wall and the rotor again put into operation. If the solution contains two materials which are sedimented out, the deposit on the top of the cylinder contains a larger percentage of the heaviest of these materials while the deposit on the bottom part of the cylindrical surface contains mostly the lighter component. Consequently by proper adjustments, the various components can be purified. Also from an analysis of this deposit as a function of the distance from the top of the cylinder an estimate of the sedimentation constants S_1 and S_2 and hence the molecular weights M_1 and M_2 of the two substances respectively can be obtained. If the solution contains an uncharged homogeneous molecular species as a solute with a sedimentation constant S, and if all this solute is deposited on the inner cylindrical wall of radius R_2, then the amount of solution F, in which the centrifuge can completely remove all of the solute per second, is approximately (*16*):

$$F = \frac{\pi L \omega^2 S}{2}\left(R_2 + R_1\right)^2. \qquad (10)$$

From this it can be seen that with the tubular centrifuges we described about 10 years ago, when we were working on this problem, one tubular centrifuge was equivalent to about 40 machines of the "quantitative type" of Fig. 6. Therefore this type of tubular centrifuge should be used when large quantities of material are to be processed.

If only very small quantities of solution are available the tubes T in Fig. 6 should be reduced in size so that they are always filled. Whenever possible, the vacuum-type centrifuge should be used because of the absence of thermal gradients. However, if

the material has a sedimentation constant above about 4 to 5 Svedberg units and only a very small amount of solution is available a rotor similar to Fig. 6 may be mounted on a small air-driven turbine shown in Fig. 8. Such apparatus is easy to make and costs only a few dollars (*16*). However, extreme care must be taken in stopping the rotor to prevent remixing. Also we have found that an artificial radial temperature gradient (decreasing from axis to periphery) introduced by focusing a hot-pointed heater on the region near the axis of the rotor tends to stabilize the sedimentation. Such thermal gradients also may be introduced by electrical means into the vacuum-type centrifuge rotors if desired, but if the pressure is below 10^{-5} mm Hg or if hydrogen at a pressure of less than a millimeter of Hg surrounds the rotor it is unnecessary. Also the vacuum-type ultracentrifuge can easily be operated between 4°C and 0°C, over which range in aqueous solution an increase of temperature increases the density, so the periphery may be made warmer than the axis of the rotor.

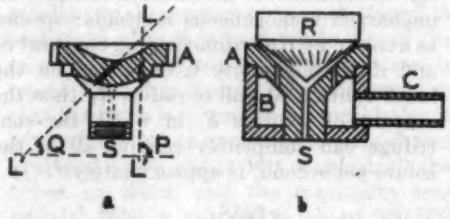


FIG. 8.—Small air-driven air-supported centrifuge.

In equation (6) it will be observed that if $V\rho = 1$ then $dr/dt = 0$ and the sedimentation stops. In other words, no sedimentation takes place if the density of the solvent ρ_d and the density ρ_p of the solute are exactly the same. Consequently, if a radial density gradient exists in a centrifuge cell over a range of densities which would include the density ρ_p of the solute, then the solute will seek a radial level in the centrifuge where the density of the solution ρ coincides with ρ_p. Clearly the sharpness of this radial level gives a measure of the homogeneity of the material being centrifuged. Also the density $\rho = 1/V$ can be determined directly. The value of the partial specific volume V obtained in this way clearly may not be the same as the value of V in dilute solution due to possible changes in hydration brought about by the material added to the solution to increase its density. In the case of proteins, the value of ρ_p in the unsolvated state is about 1.3 gm/cc although values from 1.1 to 1.5 gm/cc have been reported. Hence in order to carry out experiments with proteins, a comparatively low molecular weight inactive material must be added to the water solvent such as pure sugar.

The radial stratification of substances of different densities can be carried out very conveniently with the vacuum-type centrifuges of Fig. 1 where the process can be observed. The smallest density gradient is set by the compressibilities of the solvent and solute where a pure liquid solvent is used, but where other low molecular weight substances are added, the major portion of the density gradient can be calculated by equation (3), since equilibrium must exist before a stable density gradient is established. With the vacuum type of centrifuge rotor of Fig. 1, the radial density gradient in the centrifuge cell which contains pure water is approximately 1 per cent per cm with a rotor speed of 1,000 r.p.s. E. N. and E. B. Harvey, H. W. Beams, and others have discussed the possibility that this density gradient centrifuge method might be used for the separation of the chromosomes and other cell components. Recently E. N. Harvey (*33*) has calculated the density gradients necessary to separate certain X and Y chromosomes in man and in some animals and has concluded that they might be separated in the vacuum-type ultracentrifuge similar to that in Fig. 1. The high resolving power ultracentrifuge of Fig. 6 also would seem to be well suited to this problem of the separation and collection of the chromosomes or other cell components.

The relative displacement of the various components of the biological cell in centrifugal fields has been studied by a number of different workers with most interesting and important results. No attempt can be

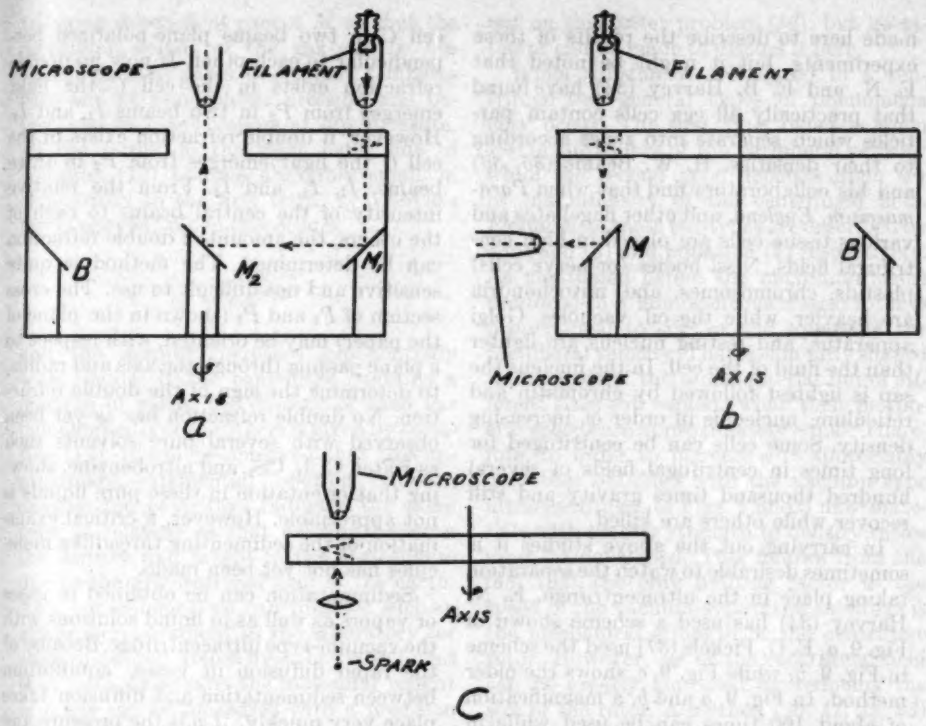


FIG. 9.—Schematic diagram of methods of viewing or photographing objects in a centrifuge by means of a microscope.

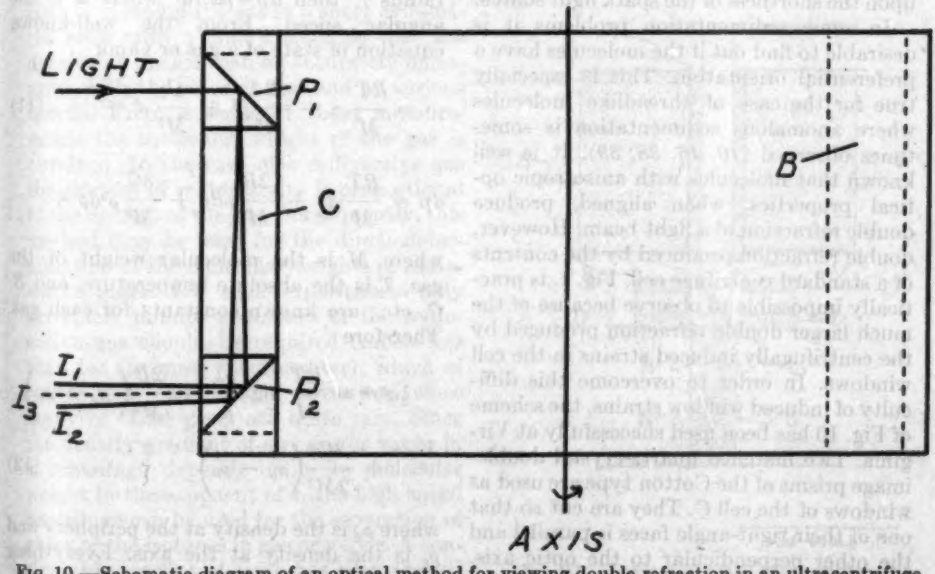


FIG. 10.—Schematic diagram of an optical method for viewing double refraction in an ultracentrifuge.

made here to describe the results of these experiments, but it might be noted that E. N. and E. B. Harvey (*34*) have found that practically all egg cells contain particles which separate into zones according to their densities. H. W. Beams (*35, 36*) and his collaborators find that when *Paramaecium*, *Euglena*, and other flagellates and various tissue cells are placed in high centrifugal fields, Nissl bodies (or nerve cells) plastids, chromosomes, and mitochondria are heavier, while the oil, vacuoles, Golgi apparatus, and resting nucleus are lighter than the fluid of the cell. In the nucleus the sap is lightest followed by chromatin and reticulum, nucleolus in order of increasing density. Some cells can be centrifuged for long times in centrifugal fields of several hundred thousand times gravity and still recover while others are killed.

In carrying out the above studies it is sometimes desirable to watch the separation taking place in the ultracentrifuge. E. N. Harvey (*34*) has used a scheme shown in Fig. 9, *a*, E. G. Pickels (*37*) used the scheme in Fig. 9, *b*, while Fig. 9, *c*, shows the older method. In Fig. 9, *a* and *b*, a magnification of about 100 times can be used while in 9, *c*, the possible magnification depends upon the shortness of the spark light source.

In some sedimentation problems it is desirable to find out if the molecules have a preferential orientation. This is especially true for the case of threadlike molecules where anomalous sedimentation is sometimes observed (*10, 16, 38, 39*). It is well known that molecules with anisotropic optical properties, when aligned, produce double refraction of a light beam. However, double refraction produced by the contents of a standard centrifuge cell, Fig. 1, is practically impossible to observe because of the much larger double refraction produced by the centrifugally induced strains in the cell windows. In order to overcome this difficulty of induced window strains, the scheme of Fig. 10 has been used successfully at Virginia. Two matched quartz-crystal double-image prisms of the Cotton type are used as windows of the cell C. They are cut so that one of their right-angle faces is parallel and the other perpendicular to the optic axis. If light falls upon P_1 it emerges into the cell C in two beams plane-polarized perpendicular to each other. If now no double refraction exists in the cell C the light emerges from P_2 in two beams I_1 and I_2. However, if double refraction exists in the cell C the light emerges from P_2 in three beams, I_1, I_2, and I_3. From the relative intensity of the central beams to each of the others, the amount of double refraction can be determined. The method is quite sensitive and not difficult to use. The cross section of P_1 and P_2 (shown in the plane of the paper) may be oriented, with respect to a plane passing through the axis and radius, to determine the sign of the double refraction. No double refraction has as yet been observed with several pure solvents such as water, CCl_4, CS_2, and nitrobenzine, showing that orientation in these pure liquids is not appreciable. However, a critical examination of the sedimenting threadlike molecules has not yet been made.

Sedimentation can be obtained in gases or vapors as well as in liquid solutions with the vacuum-type ultracentrifuge. Because of the rapid diffusion in gases, equilibrium between sedimentation and diffusion takes place very quickly. If p is the pressure and ρ the density of the gas or vapor at any radius r, then $dp = \rho\omega^2 r dr$ where ω is the angular speed. From the well-known equation of state of a gas or vapor

$$p = \frac{RT}{M}\rho + \frac{B}{M^2}\rho^2 + \frac{C}{M^3}\rho^3 + \qquad (11)$$

$$dp = \frac{RT}{M}d\rho + \frac{2B}{M^2}\rho d\rho + \frac{3C}{M^3}\rho^2 d\rho +$$

where M is the molecular weight of the gas, T is the absolute temperature, and B, C, etc., are known constants for each gas. Therefore

$$\tfrac{1}{2}\omega^2 r^2 = \frac{RT}{M}\log\frac{\rho_p}{\rho_c} + \frac{2B}{M^2}\left(\rho_p - \rho_c\right) + \frac{3C}{2M^3}\left(\rho_p^2 - \rho_c^2\right) \qquad (12)$$

where ρ_p is the density at the periphery and ρ_c is the density at the axis. Everything in equation (12) can be measured in a cen-

trifuging experiment except M so that the molecular weight of the gas can be obtained. In some gases, at ordinary temperature and at atmospheric pressure and below, and in many vapors at low pressure, all the terms on the right hand side of equation (12) become negligibly small in comparison to the first term; i.e., Boyle's law is obeyed. When this occurs,

$$\rho_p = \rho_a e^{\frac{M\omega^2 r^2}{2RT}},$$

and the density increases exponentially with radius. If the centrifuge contains a mixture of gases, the density of each gas independently of the others is given by equation (12). It will be observed that this approximate exponential relation between density and centrifuge radius is the same type of relation as that existing between the density and height in the atmosphere due to the gravitational field of the earth. As a consequence we can produce such an atmospheric gradient in the laboratory for study if desired. Recently, it has been found that, with some care, the molecular weight of a gas can be measured with a precision of the order of 0.1 per cent by the above centrifuging method. The experiment consists in spinning the gas in a short cylindrical rotor with a hollow shaft so that the density at the axis can be accurately measured, with the rotor at rest and at various speeds. From a series of these measurements the molecular weight of the gas is obtained. In the case of a radioactive gas the amount of radioactivity is proportional to the density of the gas. Consequently, this method may be used for the direct determination of the molecular weights of radioactive gases. For such experiments only extremely minute quantities of the radioactive gas should be required (much less than for the mass spectrometer), which of course is a considerable advantage since many of these gases are quite rare. Since the density gradient of any gas or vapor in a centrifuge depends upon its molecular weight in the exponent of e, the high speed centrifuge can be used for the separation of gases or the isotopes in gas or vapor. A considerable amount of work has been carried out on this latter problem (*16*), but no attempt can be made to discuss it here.

When substances are placed in a centrifuge two general types of phenomena may occur. The first type depends upon ω^2r^2 or the square of the peripheral velocity while the second type depends upon ω^2r or the intensity of the centrifugal field. Examples of these two types are described by equation (3) and equation (7) respectively. As mentioned before the maximum stress produced in a homogeneous elastic rotor is proportional to ω^2r^2 or the square of the peripheral velocity. That is, similarly shaped rotors made of the same material but of different sizes will explode when their peripheral speeds are approximately equal. Therefore, in order to increase ω^2r^2 appreciably over that obtained with the vacuum type ultracentrifuges of Figs. 1 and 3 new materials with greater strength to density ratios must be developed. This follows from the fact that the only limiting factor to the speed of the vacuum-type ultracentrifuge is the mechanical strength of the centrifuge rotor. However, ω^2r or the centrifugal field can be greatly increased by reducing the size of the rotor. A method (*40, 41, 42, 43*) for doing this is shown schematically in Fig. 11. The steel rotor R is suspended in

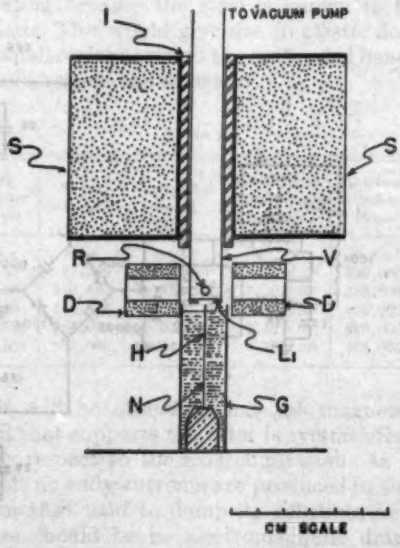


FIG. 11. Diagram of small-rotor apparatus.

the axial magnetic field of the solenoid S and is spun by a 2-phase rotating magnetic field in the two pairs of coils D. The vertical position of the steel (or other ferromagnetic) rotor is maintained by an automatic regulation of the current through the

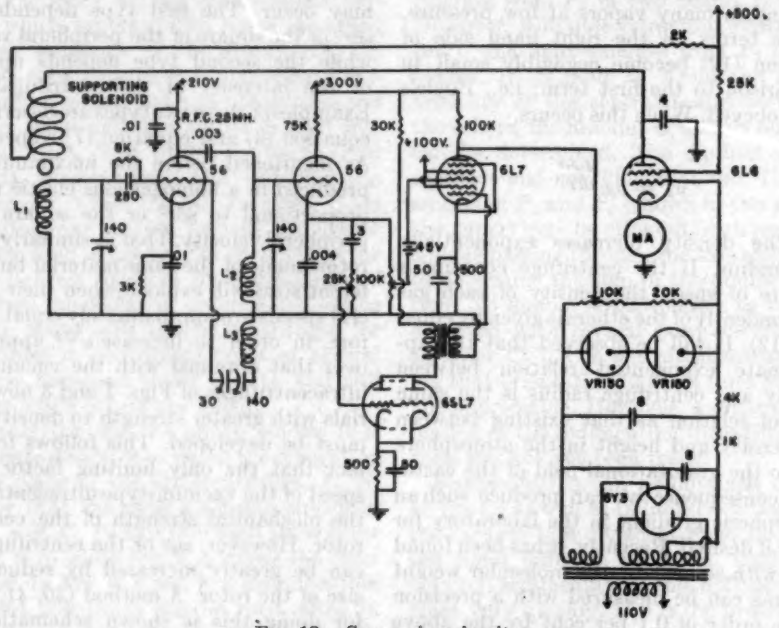


FIG. 12.—Suspension circuit.

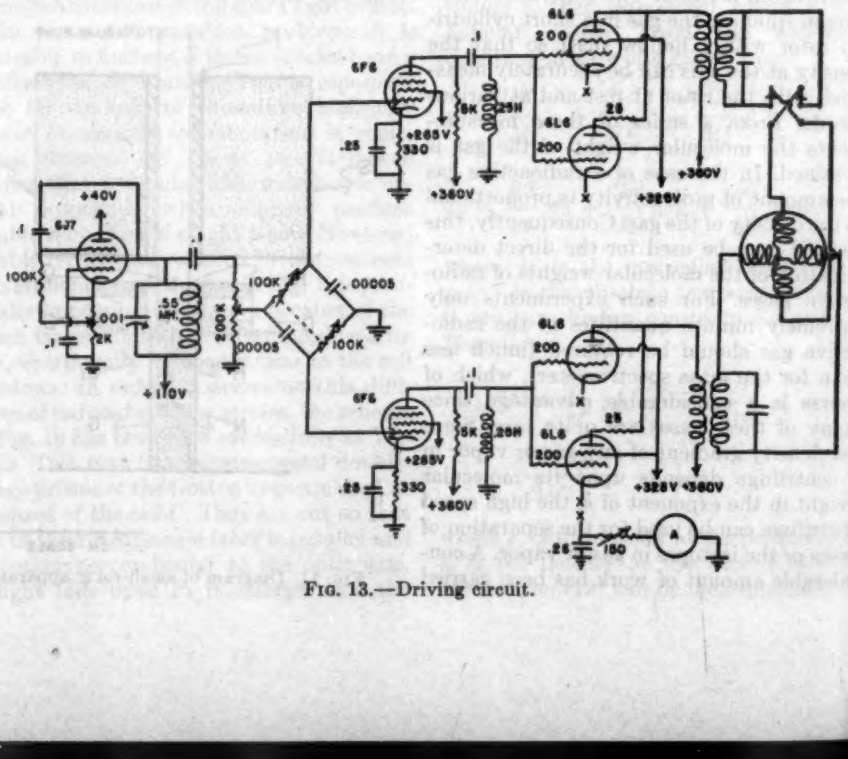


FIG. 13.—Driving circuit.

solenoid S while its horizontal position is maintained by the symmetrically diverging magnetic field of the solenoid. The small "pick up" coil L_1 is in the grid circuit of a tuned-grid tuned-plate radio-frequency oscillator, which regulates the current through S (Fig. 12). This circuit is so arranged that when the rotor R rises the current through S decreases. These current changes are such as to position accurately the rotor without observable hunting. It will be observed from Fig. 12 that this is accomplished by introducing a derivative or "anti-hunt" signal along with the signal from the pick-up coil L_1. An idea of the stability of the rotor can be obtained from the fact that no vertical motion could be observed with a 30-power microscope focused on the markings of the rotor. A small iron wire H mounted in a glass tube filled with a liquid assists in damping any horizontal motion of the rotor. The glass chamber V surrounding the rotor R was evacuated by the usual cold trap, diffusion pump, and fore pump combination. The pressures surrounding the rotor were measured by an ionization gauge. Fig. 13 shows a diagram of the driving circuit which produces the rotating magnetic field in the coils D. The output of the transition oscillator is passed through a volume control and then a phase-splitting bridge. Each of the phases is amplified and applied to the coils so that the current in the coils D are approximately 90° out of phase. The frequency was varied up to 750,000 cycles/sec, which was used for accelerating a 0.521-mm rotor. The rotor speed is measured by the arrangement schematically shown in Fig. 14. One-half of the rotor is polished and the other half darkened by dipping in dilute H_2SO_4, which had been in contact with metallic antimony. The dark layer is very thin and adheres to the rotor until the centrifugal field reaches the order of 4×10^8 gravity. Light from an incandescent lamp A is focused on the rotor R and the resulting scattered light focused on the electron multiplier photocell P. The variable output from the photocell, owing to the spin of the rotor, is amplified and fed to one pair of plates of a cathode ray oscilloscope. The frequency of the rotor is then compared with the sweep frequency applied to the other pair of plates of the oscilloscope. This sweep frequency is calibrated by a standard frequency oscillator (accurate from 0.1 to 0.01 per cent) up to 50,000 cycles/sec. Above this frequency multiples of the calibrated frequencies are used (*45*).

It is well known that, for rotors made of a given elastic material, the highest peripheral speed should be obtainable when the shape is such that the radial and tangential stresses are of constant value throughout the rotor. In practice small hardened steel rotors are difficult to grind to the shape required by the above conditions so hard-spherical steel rotors (from ball bearings) were used. Each spherical rotor had a small flat (about 1 mil) ground off of it in order to determine its axis of rotation. Table 1 gives some of the results obtained just before the rotors explode. It will be observed that the maximum peripheral speed for all of the rotors is roughly 10^5 cm/sec, which is in agreement with the theory. In the fifth column are tabulated the maximum stresses in the rotors calculated by the method of Chree (*44*), assuming the rotors are perfectly elastic. These stresses reach a maximum at the center of the rotor and fall off toward the surface. The calculated values may be somewhat larger than those actually existing because the steel is known to be plastic. This would give rise to plastic flow in small regions around the center and hence would relieve the stresses.

TABLE 1

Diameter rotor	Rotor speed	Peripheral speed	Centrifugal acceleration ×gravity	Maximum calculated stress
mm	*r.p.s.*	*cm/sec*		*lb./in.²*
3.97	77,000	9.60×10^4	4.71×10^7	410,000
2.38	123,500	9.25×10^4	7.20×10^7	385,000
1.59	211,000	1.05×10^5	1.43×10^8	496,000
.795	386,000	9.65×10^4	2.40×10^8	420,000
.521	633,000	1.04×10^5	4.28×10^8	488,000

It will be observed that the magnetic field that supports the rotor is symmetrical with respect to the axis of rotation. As a result no eddy currents are produced in the rotor that tend to damp its rotation; i.e., there should be no electromagnetic drag. With a 1/16-inch or 1.59-mm rotor spinning

at 100,000 r.p.s., the driving current in the coils D was turned off and the rotor allowed to coast while the deceleration was measured. It was found that it required about 1 hour to lose 0.1 per cent of its speed. The air pressure surrounding the rotor during these experiments was approximately 2×10^{-6} mm of Hg. The drag on the rotor due to air friction can be calculated from the relation (*43*)

$$\log_e \frac{N}{N_o} = \frac{-5p}{rd}\left(\frac{M}{2\pi RT}\right)^{1/2}\left(t - t_o\right)$$

where N_o is the number of r.p.s. at the time t_o, N is the number of r.p.s. at time t, p is the pressure in bars, d is the density of the steel (7.8 gms/cm^3), T is the absolute temperature, M is the molecular weight of the gas, and r is the radius of the spherical rotor. Substituting into this equation, it is found that practically all the observed drag can be accounted for by air friction alone, although there may be a very small residual drag due to inhomogeneities. Clearly this spinning rotor arrangement should serve as an excellent absolute pressure manometer for measuring low gas pressures.

The very small observed drag on the rotor suggested that the rotor would continue to accelerate until it reached the driving frequency. That is, during the acceleration period the rotor would operate in a way similar to that of an armature of an induction motor, but as soon as it approached closely enough to the frequency of the rotating field in the driving coils D (Fig. 11) it would shift over and run as a synchronous motor. This would be expected from the fact that the rotor is steel and should become magnetized when the rotor speed and driving frequency become nearly equal. Experiment showed that the rotor actually reaches the speed of the driving frequency and then "locks into" this frequency. It is well known that the frequency of a piezoelectric crystal will remain extremely constant if it is cut properly and connected into the proper circuit and if its temperature is held constant. The driving oscillator in the circuit of Fig. 13 was replaced by such a piezoelectric crystal oscillator with a frequency of 100,000 cycles/sec. A 1.59 mm rotor surrounded by a gas pressure of 2×10^{-6} mm Hg or less was allowed to accelerate up to the frequency of the piezoelectric crystal and "lock in" with it. In order to determine when this "locking in" takes place a signal from the piezoelectric crystal driving circuit was applied to one pair of plates of a cathode-ray oscilloscope while the signal from the electron multiplier photoelectric cell (Fig. 14) was applied to the other pair of plates of the same oscilloscope. The two signals were made to give approximately the same deflection so that when the rotor speed reached exactly the driving frequency the Lissajous figure became a stationary circle on the screen. It is interesting to note that when the rotor speed first reaches the driving frequency, a slight hunting occurs. However, this hunting damps out and as far as can be determined, completely vanishes in a few minutes. This damping of the hunting is apparently electromagnetic. Therefore, the speed of these rotors can be made as constant as the frequency of a piezoelectric crystal circuit.

The temperature of the spinning rotor has not been measured directly but an upper limit for it can be obtained by measuring the temperature of the rotor at rest in the vacuum chamber with the full power applied to the driving coils D. This was ac-

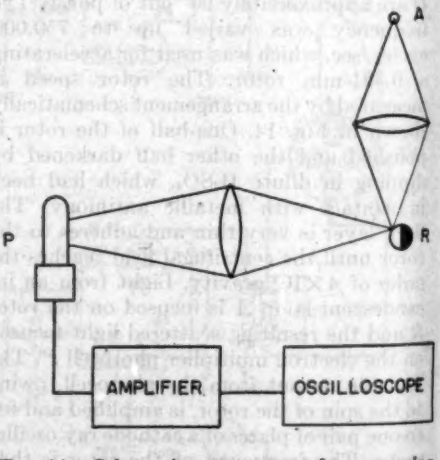


FIG. 14.—Schematic arrangement for measuring rotor speed.

complished by fastening a thermocouple made of fine wires to the rotor, then evacuating the vacuum chamber surrounding the rotor and applying the power to the coils *D*. The maximum increase in temperature was between 5° and 6°C. Therefore the rise in temperature of the spinning rotor is less than 6°C. Small mirrors have been ground on these magnetically suspended rotors without introducing appreciable added friction. Consequently rotating mirrors are available whose rotor speed not only is very high but extremely constant, and it should be possible to obtain greater precision for example in such determinations as the velocity of light. Obviously this technique of producing both very high centrifugal fields and very high and constant rotor speeds opens many new avenues for interesting investigations.

LITERATURE CITED

(1) EDDINGTON. *Mathematical theory of relativity*. Cambridge, 1924.
(2) STOKES. Cambridge Phil. Trans. **8**: 287. 1847.
(3) MASON and WEAVER. Phys. Rev. **23**: 412. 1924.
WEAVER. Phys. Rev. **27**: 499. 1926.
(4) ARCHIBALD. Phys. Rev. **53**: 746. 1938; **54**: 371. 1938.
(5) LAMM. Ark. Mat. Astr. Fys. **21B** (2). 1929; Zeitschr. Phys. Chem. **143A**: 177. 1929.
(6) FAXEN. Ark. Mat. Astr. Fys. **21B** (2). 1929.
(7) SOTEN. Proc. Phys.-Math. Soc. Japan (3) **18**: 9. 1936.
OKA, Proc. Phys.-Math. Soc. Japan (3) **19**: 1094. 1937.
(8) ARCHIBALD. [Private communication, 1946.]
(9) SVEDBERG. *Colloid chemistry*. Chemical Catalogue Co. 1928.
(10) SVEDBERG and PEDERSEN. *The ultra centrifuge*. Oxford, 1940. (See for other references.)
(11) TOLMAN. Journ. Amer. Chem. Soc. **33**: 121. 1911.
(12) MACINNES. Ann. New York Acad. Sci. **43**: 243. 1942.
(13) PICKELS. Chem. Rev. **30**: 341. 1942.
(14) PICKELS and BEAMS, J. W. Science **81**: 342. 1935.
(15) BEAMS, J. W., and PICKELS. Rev. Sci. Inst. **6**: 299. 1935.
(16) BEAMS, J. W. Journ. Appl. Phys. **8**: 795. 1937; Rev. Mod. Phys. **10**: 245. 1938; Rep. Prog. Phys. (Physical Soc.) **8**: 31. 1941. (See for other references.)
(17) BEAMS, J. W., LINKE, and SOMMER. Rev. Sci. Inst. **18**: 57. 1947.
(18) LAMM. Nova. Acta. Reg. Soc. Sci. Upsaliensis (4) **10** (6). 1937.
(19) PHILPOT. Nature **141**: 283. 1938.
(20) SVENSSON. Kolloid—Z, **87**: 181. 1939.
(21) ANDERSSON. Nature **143**: 720. 1939.
(22) BRIDGMAN and WILLIAMS. Ann. New York Acad. Sci. **43**: 195. 1942.
(23) SKARSTRON and BEAMS, J. W. Rev. Sci. Inst. **11**: 398. 1940.
(24) BEAMS, J. W. Ann. New York Acad. Sci. **43**: 177. 1942.
(25) ROTHEN. Ann. New York Acad. Sci. **43**: 299. 1942.
(26) BULL. *Physical biochemistry*. Wiley, 1943.
(27) BEAMS, J. W. Science in Progress (2) **9**: 232. Yale, 1940.
(28) ZWORKIN, MORTON, RAMBERG, HILLIER, and VANCE. *Electron optics and the electron microscope*. Wiley, 1945.
(29) BEAMS, J. W. Phys. Rev. **69**: 546A. 1946.
(30) WYCKOFF. Proc. Amer. Phil. Soc. **77**: 455. 1937; Journ. Biol. Chem. **121**: 219. 1937; **122**: 239. 1937; **124**: 585. 1938.
(31) PICKELS. Journ. Gen. Physiol. **26**: 341. 1943.
(32) MASKET. Rev. Sci. Instr. **12**: 277. 1941.
(33) HARVEY, E. N. Journ. Hered. **37**: 71. 1946.
(34) HARVEY. Journ. Franklin Inst. **214**: 1. 1932; Biol. Bull. **66**: 48. 1934; Journ. Appl. Phys. **9**: 68. 1938. (See for other references.)
(35) BEAMS, H. W., and KING. Anat. Rec. **59**: 363, 395. 1934.
(36) BEAMS, H. W. Biol. Symposia **10**: 71 1943. (See for other references.)
(37) PICKELS. Science **83**: 471. 1936.
(38) STANLEY and LAUFFER. Science **89**: 345. 1939. LAUFFER. Journ. Phys. Chem. **44**: 1137. 1940.
(39) JULLANDER. Ark. Kemi. Min. Geol. **21A** (2). 1945.
(40) HOLMES and BEAMS, J. W. Nature **140**: 30. 1937.
(41) MACHATTIE. Rev. Sci. Inst. **12**: 429. 1941.
(42) BEAMS, J. W., YOUNG, and MOORE. Journ. Appl. Phys. **17**: 886. 1947.
(43) BEAMS, J. W., and YOUNG. Phys. Rev. **71**: 131. 1947.
(44) CHREE. Proc. Roy. Soc. **58**: 39. 1895.
(45) SHAPIRO and BUTT. Rev. Sci. Inst. **8**: 34. 1937.

ENTOMOLOGY.—*A new* Schematiza *on* Cordia *in Trinidad* (*Coleoptera: Chrysomelidae*).[1]

H. S. BARBER, Bureau of Entomology and Plant Quarantine.

A third request for specific identification of a galerucine beetle destructive to *Cordia macrostachya* in Trinidad requires proposal of a new specific name. The two previous samples received in 1944 and 1945 were compared with old samples of *Schematiza lateralis* presented to the National Museum by Godman and Salvin from the "Biologia" collection and were found distinct as herein noted; but parallel samples sent for identification from Trinidad to England were reported under the name of that Central American species, which displays rather distinctive characters. This tropical genus may be as complex, and the species as difficult to distinguish, as is our closely related genus *Galerucella*, in which similar forms or species seem more easily recognized by their host associations than by any characters visible in preserved specimens. Such data for tropical forms however, are, rarely available.

The genonym *Schematiza* was proposed by Chevrolat, 1837 (Catalt. Coleopt. Coll. Dejean, 3d ed., p. 401; p. 377 of the "1833" edition), including one previously described species, *Lycus laevigatus* Fabricius, 1801, and *nomina nuda* for five other species. Its citation as a *nomen nudum* by Sherborn, 1930 (Index Animalium, p. 5789), and by Neave, 1930 (Nomenclator Zoologicus 4: 130), seems misleading. Guérin, 1844 (Icon. Règne Animal, Ins., p. 303), adopted the name from the Dejean catalogue but stated his uncertainty as to generic relationship of the five species he named as new, with the species in the Dejean collection. Chevrolat, 1845 (*in* D'Orbigny, Dict. Hist. Nat. 6: 5), cites *C. marginata* Fabricius as type of *Schematiza*, but as this name was not originally included and no such species seems traceable in recent catalogues, it must have been a *lapsus*. Blanchard, 1845 (Hist. Ins. 2: 190), offers a description of *Schematiza* Chevrolat without mention of any species. Chevrolat, 1848 (*in* D'Orbigny, Dict. Hist. Nat. 11: 413), again treats the genus with *Lycus laevigatus* Fabricius and the five *nomina nuda* as originally listed. The number of described species grew to 19 listed by Gemminger and Harold, 1876, in the Munich catalogue and 32 listed by Weise, 1924, in the Junk catalogue, the former ascribing the genus to Blanchard, 1845, and the latter to Guérin, 1844, with *lycoides* Guérin, 1844, cited as genotype. Under the Code, *Schematiza* Chevrolat is valid in Dejean, 1837, and *Lycus laevigatus* Fabricius, 1801, the only described species there available and automatically its genotype, is now designated type of the genus.

Schematiza laevigata (Fabricius)

Lycus laevigatus Fabricius, Syst. El. 2: 117. 1801.
Homalisus laevigatus Schoenherr, Syn. Ins. 1 (pt. 3): 77. 1817.
Schematiza axillaris Dejean, Cat. Coleopt. Coll. Dejean, 3d ed.: 402. 1837.
?*Schematiza axillaris* Guérin, Icon. Règne. Animal, Insects: 304. 1844.

This species seems to be lost from recent catalogues. It was described as black with thoracic margin and a basal elytral line rusty, the elytra smooth, the body small, the antennae compressed and wholly black, and the habitat data as "in America meridionalis. D. Smidt. Mus. D. Lund." The name *laevigatus* Fabricius, 1801, is listed in *Homalisus* by Schoenherr, 1817, and by Sturm, 1826 (Catal. meiner Ins. Sammlung, p. 153), the latter changing its habitat to Brazil. I have failed to trace the name beyond 1837 where *axillaris* Dejean attained nomenclatorial status by its publication as a new name for *laevigatus* Fabricius, and 1844, where Guérin suggested that his "new species" *axillaris* from Cayenne might be conspecific with *laevigatus* F. as now again seems probable. A specimen from the Wickham collection labeled "Bartica, Demerara, R. J. Crew" agrees with the descriptions of both *laevigatus* and *axillaris*. Mequignon, 1934 (Bull. Soc. Ent. France 39: 171–172), explains that Smidt resided in the Danish West Indian island of St. Thomas and assumes this to be the type locality of two problematic species of *Chelonarium*, but in similar problems involving types from Smidt the ports made en route to and from his objective must be considered. Delay in these ports might have per-

[1] Received March 28, 1947.

mitted him to collect there for the museums of Messrs. Lund and Sehestedt.

Schematiza lateralis Jacoby

Jacoby, 1887 (Biol. Centr.-Amer., Coleopt., 6 (pt. 1): 498), adopts *Schematiza* (Chevrolat) Blanchard, 1845, with comments on included forms so similar to *Galerucella* as to suggest assignment there. Among his eight new species *S. lateralis* is described (p. 499) from "Mexico, Oaxaca (Salle), and Panama, Volcán de Chiriquí, Bugaba (Champion)," mentioning many specimens but designating no type locality or holotype. Four of the specimens collected by Champion on the Volcán de Chiriquí were presented to the United States National Museum. One of these specimens does not agree with Jacoby's description and is obviously specifically distinct from the other three. Lest the Oaxaca cotype be also found specifically distinct, the type locality of *lateralis* is now designated as the Volcán de Chiriquí. In these three cotypes a rather strong costa, prominent enough above the submarginal sulcus to obscure the marginal costa in direct dorsal aspect, runs almost straight from humerus to apical fifth of elytra where it curves strongly inward ending near middle of apex. This costa, the pale submarginal elytral vitta, the shape of the pronotum, the black median pronotal vitta and the narrower, more parallel form are peculiarities of *lateralis* not found in the species from Trinidad which has been misidentified as *lateralis* but which may be known as follows:

Schematiza cordiae, n. sp.

Opaque, fuscous, depressed, elongate oval, sides arcuate, length 6–7.5 mm, width 3–3.6 mm.

Pronotum twice as wide as long, sides expanded and strongly arcuate, surface broadly impressed at lateral fourths and narrowly over occiput. Elytra smooth, evenly convex from within the prominent marginal costa to the elevated sutural costa, showing no vestige of submarginal costa characteristic of *lateralis;* sculpture and vestiture fine and dense. Antennae slightly thickened near middle, tapering apically, not flattened. Sexes extremely similar.

Described from 16 specimens labeled "on black sage, St. Augustine, Trinidad, B. W. I. May 2, 1944. A. M. Adamson, collector, 4070."

Host plant.—Cordia macrostachya.

Holotype male and 12 paratypes, U.S.N.M. no. 58362.

Three paratypes returned to F. J. Simmonds in Trinidad. A similar sample now referred to this species consists of 31 specimens labeled "on *Cordia cylindrostachya*, Caracas, D. F., Venezuela, Oct. 4, 1939, C. H. Ballou, No. 934." Eight specimens from St. Vincent seem to represent a very closely related species.

ENTOMOLOGY.—*New North American species and new assignments in the genus* Chionodes (*Lepidoptera: Gelechiidae*).[1]

J. F. GATES CLARKE, Bureau of Entomology and Plant Quarantine.

The purpose of this paper is to assign certain species of North American gelechiids, the generic position of which was not determined by Busck, and to describe some of a large number of hitherto unnamed species in this family that have been accumulating at the United States National Museum. In Busck's paper[2] he did not recognize some of Annette F. Braun's and E. Meyrick's species and therefore did not assign them to their proper genera. I have examined some of these and will assign them to the appropriate genera in this and subsequent papers.

Genus **Chionodes** Hübner

Chionodes nigrobarbata (Braun), n. comb.

Gelechia nigrobarbata Braun, Can. Ent. 57: 125. 1925.

Chionodes occlusa (Braun), n. comb.

Gelechia occlusa Braun, Can. Ent. 57: 126. 1925.

Chionodes permacta (Braun), n. comb.

Gelechia permacta Braun, Can. Ent. 57: 126. 1925.

Chionodes asema, n. sp.

Figs. 6–6c, 15

Labial palpus with second segment ocherous

[1] Received February 4, 1947.

[2] BUSCK, A., *Restriction of the genus* Gelechia *(Lepidoptera: Gelechiidae), with descriptions of new species.* Proc. U. S. Nat. Mus. 86: 574. 1939.

white inwardly, blackish fuscous exteriorly and in the brush; third segment mixed blackish fuscous and ocherous-white; tip ocherous-white. Antenna blackish fuscous annulated with pale ocherous. Head and thorax pale yellowish brown, mixed with a few scattered black and fuscous scales; base of tegula blackish fuscous. Forewing ground color sordid white, so heavily overlaid with pale yellow-brown and blackish-fuscous markings as to be obscured except in some specimens in which the ground color is pronounced along costa; at basal fourth an outwardly oblique, blackish-fuscous fascia extends almost to dorsum at basal third; a spur of this fascia branches off and extends almost to middle of cell; from the end of cell to tornus is another outwardly oblique, blackish-fuscous fascia; from costa, at apical third a narrow, S-shaped, pale line extends completely across wing; preceding the pale line is a fuscous spot on costa; beyond this line is a blackish-fuscous shading; cilia pale yellowish brown. Hind wing light gray basally shading to blackish fuscous at apex; cilia fuscous, first and second legs ocherous white inwardly, banded with blackish fuscous outwardly. Abdomen fuscous above, buff beneath, shaded with fuscous.

Male genitalia.—Harpes asymmetrical. Right harpe slender and, with its terminal setae, nearly attaining apex of uncus; at base is a strong angulate process and near distal end is a small, sharp ventrolateral tooth; left harpe long, slender, curved, terminating in a sharp point. Gnathos strong, angulate. Aedeagus slightly more than twice as long as right harpe, the stalk half as long as the stout distal portion; from side, near terminal end, a strong sclerotized process divided at its outer end.

Female genitalia.—Ostium wide. Ductus bursae broad, sclerotized for nearly its entire length. Inception of ductus seminalis at posterior end of bursa copulatrix; in posterior part of bursa are numerous small scobinations; signum a subquadrate plate with several sharp teeth at the anterior and posterior ends.

Alar expanse, 12–16 mm.

Type.—U.S.N.M. no. 58234.

Type locality.—Putnam County, Ill.

Remarks.—Described from the ♂ type and five ♂ and two ♀ paratypes as follows: ♂ type, and 1 ♂ paratype, Putnam County, Ill. (29-VI and 15-VII-1941, Murray O. Glenn); 2 ♂ ♂, 2 ♀ ♀, Oak Station, Allegheny County, Pa. (29-VII-1911, 4-VII-1909, 5-VII-1908, Fred Marloff); 3 ♂ ♂, Pittsburgh, Pa. (2-30-VII-1906, Henry Engel).

Paratypes in the United States National Museum, the British Museum, and the Murray O. Glenn collection.

This species is strikingly similar to *C. negundella* (Heinrich), but can be distinguished from it by the large basal process and small terminal spur of the right harpe, and by the signum, which is twice as large in *asema* and lacks the toothed edge of the signum of *negundella*.

Chionodes iridescens, n. sp.
Figs. 1–1a, 12

Labial palpus with second segment ocherous-white shading to ocherous in the brush; the entire length of second segment overlaid with black scaling above; third segment black except base and a few scattered scales, which are pale ocherous. Antenna black, annulated with sordid whitish. Face buff. Head, thorax, and forewing black with a strong purple iridescence; at apical third of forewing is a conspicuous, but small, buff spot; cilia gray. Hind wing grayish fuscous, darker toward margins; cilia gray. Underside of fore and hind wings sooty. First and second legs black with a few scattered buff scales; tarsi annulated with buff; third leg black exteriorly, buff inwardly; tarsi buff and black. Abdomen shining fuscous above, iridescent black beneath.

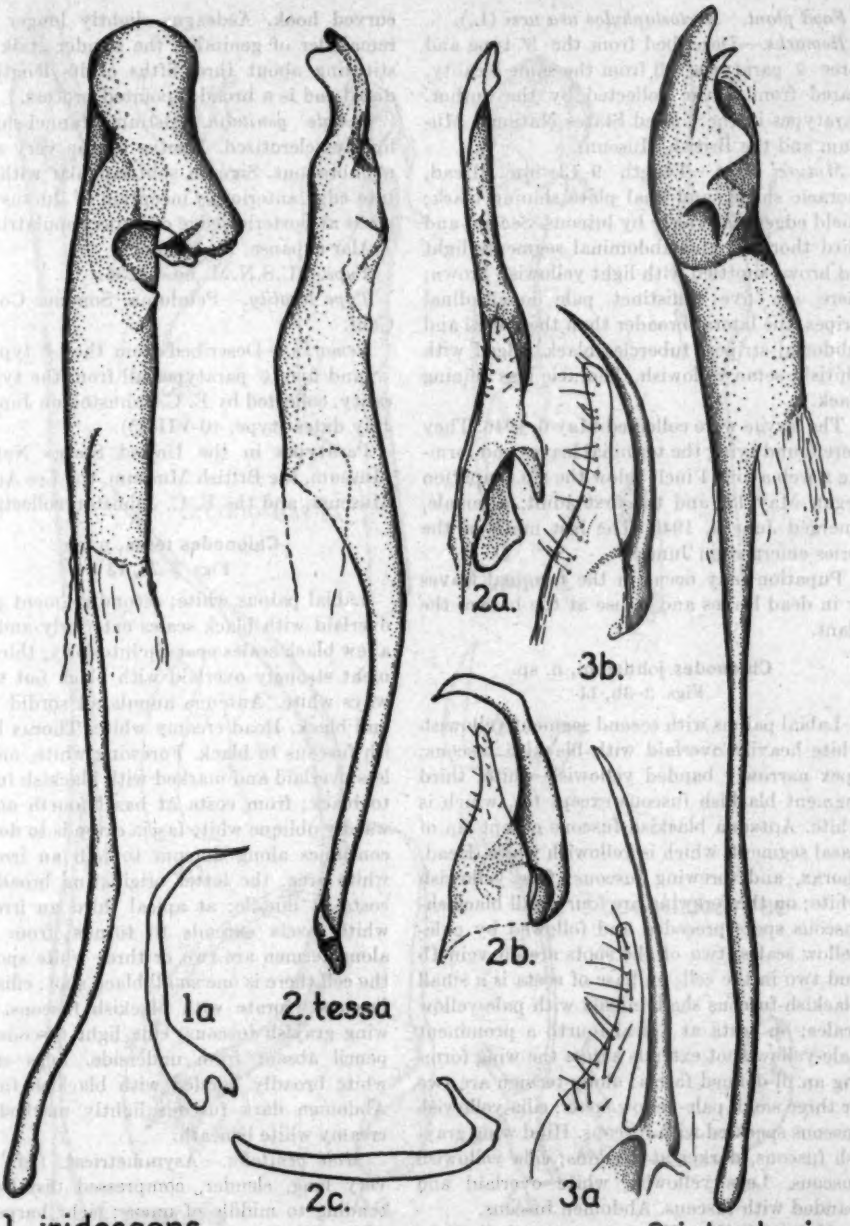
Male genitalia.—Harpes nearly symmetrical, about as long as the uncus, slender, curved. Gnathos long, slender, curved. Uncus large, hood-shaped, as broad as long. Aedeagus as long as the uncus, tegumen, and vinculum combined; slender stalk of aedeagus slightly longer than the enlarged distal part; from side, near end of aedeagus, a large winglike, bifid structure.

Female genitalia.—Ostium broad, opening into the broad, flattened sclerotized ductus bursae, which narrows as it joins the bursa copulatrix; inception of ductus seminalis from an evagination on the posterior left side of the bursa; signum a large irregular plate with two small thornlike processes from anterior and posterior ends.

Alar expanse, 16–17 mm.

Type.—U.S.N.M. no. 58235.

Type locality.—American Lake, Pierce County, Wash.



FIGS. 1–1a.—*Chionodes iridescens*, n. sp.: 1, Lateral view of aedeagus; 1*a*, left harpe, lateral aspect.
FIGS. 2–2c.—*Chionodes tessa*, n. sp.: 2, Lateral view of aedeagus; 2*a*, left harpe; 2*b*, right harpe; 2*c*, gnathos.
FIGS. 3–3b.—*Chionodes johnstoni*, n. sp.: 3, Lateral view of aedeagus; 3*a*, left harpe; 3*b*, right harpe.

Food plant.—*Arctostaphylos uva ursi* (L.).

Remarks.—Described from the ♂ type and three ♀ paratypes, all from the same locality, reared from larvae collected by the author. Paratypes in the United States National Museum and the British Museum.

Mature larva.—Length 9–13 mm. Head, thoracic shield, and anal plate shining black; shield edged anteriorly by luteous. Second and third thoracic, and abdominal segments light red brown mottled with light yellowish brown; there are five indistinct pale longitudinal stripes, the lateral broader than the dorsal and subdorsal stripes; tubercles black, edged with whitish; setae yellowish. Thoracic legs shining black.

The larvae were collected May 6, 1946. They were found tying the terminal leaves and forming a web about 1 inch below the tip. Pupation began May 12, and the first adult, a female, emerged June 3, 1946. The last moth of the series emerged on June 8.

Pupation may occur in the terminal leaves or in dead leaves and refuse at the base of the plant.

Chionodes johnstoni, n. sp.
Figs. 3–3b, 11

Labial palpus with second segment yellowish white heavily overlaid with blackish fuscous; apex narrowly banded yellowish white; third segment blackish fuscous except tip, which is white. Antenna blackish fuscous except tip of basal segment, which is yellowish white. Head, thorax, and forewing fuscous; face yellowish white; on the forewing are four small blackish-fuscous spots preceded and followed by pale-yellow scales; two of the spots are on vein 1b and two in the cell; on base of costa is a small blackish-fuscous shade mixed with pale-yellow scales; on costa at apical fourth a prominent pale-yellow spot extends across the wing forming an ill-defined fascia; along termen are two or three small pale-yellow spots; cilia yellowish fuscous speckled with fuscous. Hind wing grayish fuscous, darker at margins; cilia yellowish fuscous. Legs yellowish white overlaid and banded with fuscous. Abdomen fuscous.

Male genitalia.—Harpes asymmetrical. Right harpe slightly curved, sharply pointed, exceeding tegumen; basal process slightly more than two-thirds the length of harpe; left harpe about one-third longer than right harpe, slightly curved, bluntly pointed. Gnathos a strong, curved hook. Aedeagus slightly longer than remainder of genitalia, the slender stalk constituting about three-fifths of its length; at distal end is a broad 3-pointed process.

Female genitalia.—Ostium funnel-shaped, lightly sclerotized. Ductus bursae very short, membranous. Signum subtriangular with dentate edge anteriorly; inception of ductus seminalis at posterior edge of bursa copulatrix.

Alar expanse, 11–16 mm.

Type.—U.S.N.M. no. 58236.

Type locality.—Petaluma, Sonoma County, Calif.

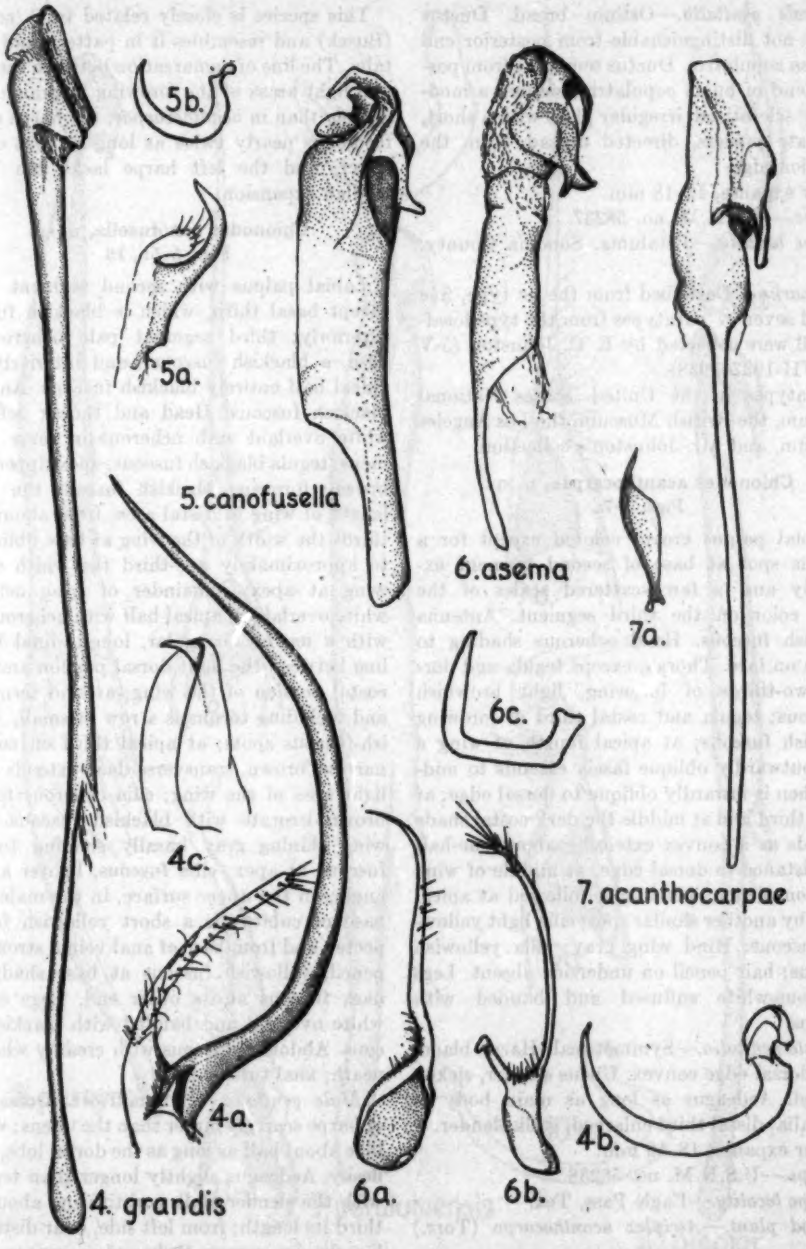
Remarks.—Described from the ♂ type, six ♂ and five ♀ paratypes all from the type locality, collected by E. C. Johnston on June ann July dates (type, 10-VII-35).

Paratypes in the United States National Museum, the British Museum, the Los Angeles Museum, and the E. C. Johnston collection.

Chionodes tessa, n. sp.
Figs. 2–2c, 13

Labial palpus white; second segment partly overlaid with black scales exteriorly and with a few black scales near tip interiorly; third segment strongly overlaid with black but tip always white. Antenna annulated sordid white and black. Head creamy white. Thorax blackish fuscous to black. Forewing white, more or less overlaid and marked with blackish fuscous to black; from costa at basal fourth an outwardly oblique white fascia extends to dorsum, continues along dorsum to join an irregular white area, the latter originating broadly on costa at middle; at apical third an irregular white fascia extends to tornus; from apex, along termen are two or three white spots; in the cell there is one small black spot; cilia light fuscous irrorate with blackish fuscous. Hind wing grayish fuscous; cilia light fuscous; hair pencil absent from underside. Legs creamy white broadly banded with blackish fuscous. Abdomen dark fuscous lightly marked with creamy white beneath.

Male genitalia.—Asymmetrical. Left harpe very long, slender, compressed distally, extending to middle of uncus; right harpe two-fifths the length of the left harpe, sharply curved ventrad. Gnathos nearly as long as uncus, sharply curved basally then nearly straight. Aedeagus as long as uncus and tegumen combined; proximal end bifid; distal end and a lateral extension, spoon-shaped.



FIGS. 4–4c.—*Chionodes grandis*, n. sp.: 4, Lateral view of aedeagus; 4*a*, right harpe; 4*b*, gnathos; 4*c*, ventrolateral aspect of uncus, in outline, to show caudal hook.
FIGS. 5–5b.—*Chionodes canofusella*, n. sp.: 5, Lateral view of aedeagus; 5*a*, left harpe; 5*b*, gnathos.
FIGS. 6–6c.—*Chionodes asema*, n. sp.: 6, Lateral view of aedeagus; 6*a*, left harpe; 6*b*, right harpe; 6*c*, gnathos.
FIGS. 7–7a.—*Chionodes acanthocarpae*, n. sp.: 7, Lateral view of aedeagus; 7*a*, left harpe.

Female genitalia.—Ostium broad. Ductus bursae not distinguishable from posterior end of bursa copulatrix. Ductus seminalis from posterior end of bursa copulatrix; signum a moderately sclerotized, irregular plate with a short, spatulate process, directed dorsad, from the posterior edge.

Alar expanse, 12–18 mm.

Type.—U.S.N.M. no. 58237.

Type locality.—Petaluma, Sonoma County, Calif.

Remarks.—Described from the ♂ type, five ♂ and seven ♀ paratypes from the type locality. All were collected by E. C. Johnston (5-V to 1-VII-1937–1938).

Paratypes in the United States National Museum, the British Museum, the Los Angeles Museum, and Mr. Johnston's collection.

Chionodes acanthocarpae, n. sp.
Figs. 7–7a

Labial palpus cream colored except for a fuscous spot at base of second segment exteriorly and a few scattered scales of the same color on the third segment. Antenna blackish fuscous. Head ocherous shading to cream on face. Thorax, except tegula and dorsal two-thirds of forewing, light brownish ocherous; tegula and costal third of forewing blackish fuscous; at apical fourth of wing a buff outwardly oblique fascia extends to middle, then is inwardly oblique to dorsal edge; at basal third and at middle the dark costal shade extends as a convex extension about one-half the distance to dorsal edge; at middle of wing is a conspicuous black spot followed at apical third by another similar spot; cilia light yellowish fuscous. Hind wing gray; cilia yellowish fuscous; hair pencil on underside absent. Legs ocherous-white suffused and banded with fuscous.

Male genitalia.—Symmetrical. Harpe blade-like, dorsal edge convex. Uncus slender, sickle-shaped. Aedeagus as long as main body of genitalia; distal third enlarged, stalk slender.

Alar expanse, 18–19 mm.

Type.—U.S.N.M. no. 58238.

Type locality.—Eagle Pass, Tex.

Food plant.—*Atriplex acanthocarpa* (Torr.) Wats.

Remarks.—Described from the ♂ type (18-V-1938, L. F. Hitchcock) and one ♂ paratype from San Diego, Calif. (12-IX-1919, Karl R. Coolidge).

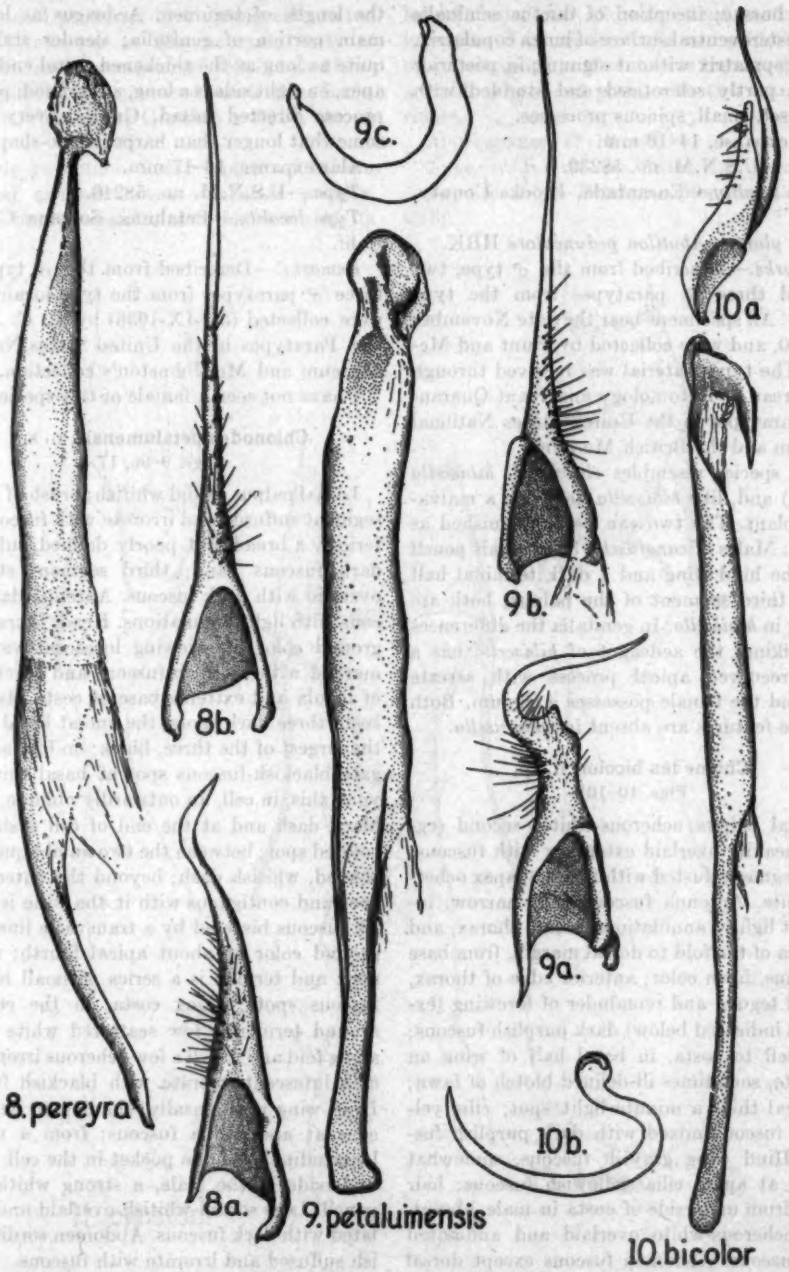
This species is closely related to *C. coticola* (Busck) and resembles it in pattern and genitalia. The line of demarcation between the dark and light areas of the forewing is straighter in *coticola* than in *acanthocarpae*, the harpe of the former is nearly twice as long as that of the latter, and the left harpe lacks the broad median expansion.

Chionodes canofusella, n. sp.
Figs. 5–5b, 16

Labial palpus with second segment white except basal third, which is blackish fuscous exteriorly; third segment pale ocherous at base, a blackish fuscous band interiorly and apical half entirely blackish fuscous. Antenna blackish fuscous. Head and thorax ocherous white overlaid with ocherous in some specimens; tegula blackish fuscous, apex tipped with brown; forewing blackish fuscous the whole length of wing in costal area, from about two-thirds the width of the wing at base obliquely, to approximately one-third the width of the wing at apex; remainder of wing ocherous white overlaid in apical half with ocherous and with a narrow, irregular, longitudinal brown line between the light dorsal portion and dark costal portion of the wing; around termen to and including tornus is a row of small, blackish-fuscous spots; at apical third on costa, a narrow, brown, transverse dash extends to the light area of the wing; cilia ocherous to light brown irrorate with blackish fuscous. Hind wing shining gray basally shading to dark fuscous at apex; cilia fuscous, lighter at anal angle; on the upper surface, in the male, from base of cubitus is a short yellowish fuscous pecten and from base of anal vein a strong hair pencil, yellowish fuscous at base shading to dark fuscous at its outer end. Legs creamy white overlaid and banded with blackish fuscous. Abdomen fuscous with creamy white beneath; anal tuft white.

Male genitalia.—Symmetrical. Dorsal lobe of harpe scarcely larger than the uncus; ventral lobe about half as long as the dorsal lobe, weak, fleshy. Aedeagus slightly longer than tegumen with the slender stalk constituting about one-third its length; from left side, near distal end, is a slender, reverse S-shaped process and from apex a short spur, curved cephalad. Uncus a strongly curved hook.

Female genitalia.—Ostium broad, funnel-shaped, narrowing to the short, membranous



FIGS. 8–8b.—*Chionodes pereyra*, n. sp.: 8, Lateral view of aedeagus; 8*a*, left harpe; 8*b*, right harpe.
FIGS. 9–9c.—*Chionodes petalumensis*, n. sp.: 9, Lateral view of aedeagus; 9*a*, left harpe; 9*b*, right harpe; 9*c*, gnathos.
FIGS. 10–10b.—*Chionodes bicolor*, n. sp.: 10, Lateral view of aedeagus; 10*a*, right harpe; 10*b*, gnathos.

ductus bursae; inception of ductus seminalis from posteroventral surface of bursa copulatrix. Bursa copulatrix without signum; in posterior portion partly sclerotized and studded with closely set, small, spinous processes.

Alar expanse, 14–16 mm.

Type.—U.S.N.M. no. 58239.

Type locality.—Encantada, Brooks County, Tex.

Food plant.—*Abutilon pedunculare* HBK.

Remarks.—Described from the ♂ type, two ♂, and three ♀ paratypes from the type locality. All specimens bear the date November 28, 1940, and were collected by Hunt and McGarr. The type material was received through the Bureau of Entomology and Plant Quarantine. Paratypes in the United States National Museum and the British Museum.

This species resembles closely *C. hibiscella* (Busck) and, like *hibiscella*, feeds on a malvaceous plant. The two can be distinguished as follows: Males of *canofusella* have a hair pencil from the hind wing and a dark terminal half of the third segment of the palpus; both are lacking in *hibiscella*. In genitalia the differences are striking; the aedeagus of *hibiscella* has a large, recurved apical process with serrate edge and the female possesses a signum. Both of these features are absent in *canofusella*.

Chionodes bicolor, n. sp.
Figs. 10–10b

Labial palpus ocherous-white; second segment heavily overlaid exteriorly with fuscous; third segment dusted with fuscous; apex ocherous-white. Antenna fuscous with narrow, indistinct lighter annulations. Head, thorax, and the area of the fold to dorsal margin, from base to tornus, fawn color; anterior edge of thorax, base of tegula, and remainder of forewing (except as indicated below) dark purplish fuscous; from cell to costa, in basal half of wing an elongate, sometimes ill-defined blotch of fawn; at apical third a minute light spot; cilia yellowish fuscous mixed with dark purplish fuscous. Hind wing grayish fuscous, somewhat darker at apex; cilia yellowish fuscous; hair pencil from underside of costa in male, absent. Legs ocherous-white overlaid and annulated with fuscous. Abdomen fuscous except dorsal surface of first three segments, which is sordid yellow.

Male genitalia.—Symmetrical. Dorsal arm of harpe slender, sharply pointed, four-fifths the length of tegumen. Aedeagus as long as main portion of genitalia; slender stalk not quite as long as the thickened distal end; from apex, on right side is a long, sclerotized, pointed process directed basad. Gnathos very large, somewhat longer than harpe, sickle-shaped.

Alar expanse, 16–17 mm.

Type.—U.S.N.M. no. 58240.

Type locality.—Petaluma, Sonoma County, Calif.

Remarks.—Described from the ♂ type and three ♂ paratypes from the type locality. All were collected (5-9-IX-1936) by E. C. Johnston. Paratypes in the United States National Museum and Mr. Johnston's collection.

I have not seen a female of this species.

Chionodes petalumensis, n. sp.
Figs. 9–9c, 17

Labial palpus sordid whitish; brush of second segment suffused and irrorate with fuscous; exteriorly a broad, but poorly defined, subapical dark fuscous band; third segment strongly overlaid with dark fuscous. Antenna dark fuscous with light annulations. Head, thorax, and ground color of forewing lutescent variously marked with blackish fuscous and black; base of tegula and extreme base of costa black; on costa three dark spots, the one at basal fourth the largest of the three, black; on fold an elongate blackish-fuscous spot at basal third; beyond this, in cell, an outwardly oblique, short, black dash and at the end of cell a similarly colored spot; between the two an oblique, tear-shaped, whitish dash; beyond the outer discal spot and contiguous with it the wing is blackish fuscous bisected by a transverse line of the ground color at about apical fourth; around apex and termen is a series of small blackish fuscous spots; along costa, in the cell and around termen a few scattered white scales; along fold and in cell a few ocherous irrorations; cilia lutescent irrorate with blackish fuscous. Hind wing gray basally, shading to dark fuscous at apex; cilia fuscous; from a narrow, longitudinal, oblique pocket in the cell, on the underside in the male, a strong whitish hair pencil. Legs sordid whitish overlaid and annulated with dark fuscous. Abdomen sordid whitish suffused and irrorate with fuscous.

Male genitalia.—Asymmetrical. Left harpe broad with the ventrodistal corner produced into a long, slender process sharply curved dorsad; right harpe as long as tegumen, stout

basally, and produced into a long attenuated process. Aedeagus as long as main body of genitalia; stalk comprising basal two-fifths; near distal end a pronounced constriction forming a knoblike apex. Gnathos a thick, strong hook.

Female genitalia.—Ventral plate of ostium produced caudad into a spatulate process. Ductus bursae membranous for most of its length, broadened and sclerotized posteriorly; inception of ductus seminalis near posterior end of membranous part of ductus bursae. Signum a small sclerotized plate with two transverse ridges.

Alar expanse, 17–19 mm.

Type.—U.S.N.M. no. 58241.

Type locality.—Petaluma, Sonoma County, Calif.

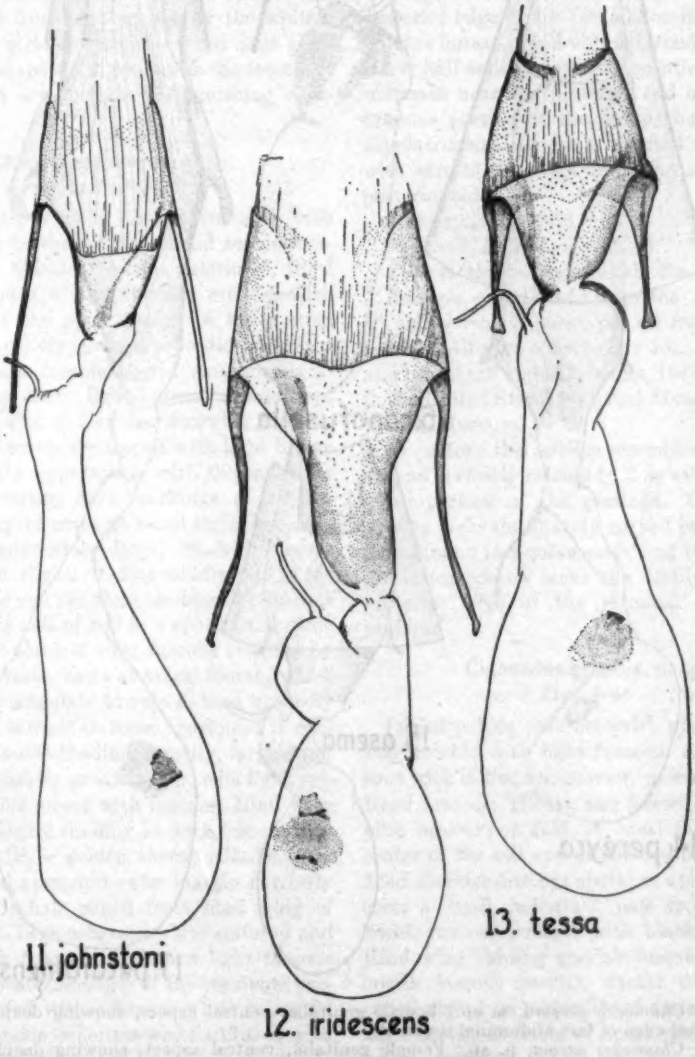


FIG. 11.—*Chionodes johnstoni*, n. sp.: Female genitalia, ventral aspect.
FIG. 12.—*Chionodes iridescens*, n. sp.: Female genitalia, ventral aspect.
FIG. 13.—*Chionodes tessa*, n. sp.: Female genitalia, ventral aspect.

FIG. 14.—*Chionodes pereyra*, n. sp.: Female genitalia, ventral aspect, showing deeply excavated posteroventral edge of last abdominal segment.
FIG. 15.—*Chionodes asema*, n. sp.: Female genitalia, ventral aspect, showing deeply excavated posteroventral edge of last abdominal segment.
FIG. 16.—*Chionodes canofusella*, n. sp.: Female genitalia, ventral aspect.
FIG. 17.—*Chionodes petalumensis*, n. sp.: Female genitalia, ventral aspect.

Remarks.—Described from the ♂ type, eight ♂ and two ♀ paratypes as follows: Petaluma, 6♂ ♂, ♀ (29-VIII-1939, E. C. Johnston); Victoria, British Columbia, ♂, 2♀ ♀ (2-IV-21, E. H. Blackmore; 20-VII-21, 15-VII-22, W. R. Carter). Paratypes in the United States National Museum, the British Museum, and in Mr. Johnston's collection.

This species might be confused with *C. bicostomaculella* (Chambers) but is at once distinguishable from that species by the whitish dash in the cell. The asymmetrical male genitalia and the spatulate process in the female of *petalumensis* are further distinguishing characters.

Chionodes pereyra, n. sp.

Figs. 8–8b, 14

Labial palpus pale luteous suffused with light brown in the brush; second segment irrorate with blackish fuscous exteriorly; third segment almost wholly overlaid with blackish fuscous, but the apex always of the ground color. Head ocherous-white to sordid white, the scales brown to fuscous-tipped. Antenna blackish fuscous with light-brown annulations. Ground color of thorax and forewing luteous; most of the scales are tipped with light brown presenting the appearance, with the exception of the contrasting dark markings, of a light-brown wing; on costa at basal third is a conspicuous, moderately large, blackish-fuscous spot; on fold, slightly before middle, and in the center of the cell are elongate blackish-fuscous spots; at the end of cell is a spot of the same color; apical third of wing fuscous bisected by a pale transverse fascia at apical fourth, which is outwardly angulate to vein 6, then inwardly angulate to tornus; in some specimens is considerable fuscous shading dorsally, largely obscuring the lighter ground color; cilia light yellowish fuscous mixed with fuscous. Hind wing pale gray basally shading to dark fuscous apically and with a golden sheen; cilia fuscous, those around apex and outer margin narrowly white-tipped; hair pencil from hind wing of male absent. Legs ocherous-white suffused and banded with fuscous. Abdomen light fuscous above, the posterior edges of the segments narrowly bordered with ocherous-white to sordid white; underside ocherous-white suffused with fuscous posteriorly.

Male genitalia.—Asymmetrical. Left harpe broad basally tapering to a sharply pointed curved tip; right harpe slender, slightly longer than tegumen and produced into a fine fleshy point. Aedeagus nearly twice as long as tegumen, slender, the stalk comprising about basal third. Distal end constricted just before apex, the latter dilated. Gnathos a moderately weak hook.

Female genitalia.—Ostium broad, opening at the edge of the deep median excavation on the posterior edge of the last abdominal segment. Ductus bursae broad and flattened in the posterior half and sclerotized, inception of ductus seminalis near the posterior end of the membranous portion of the ductus bursae. Bursa copulatrix small; signum a small round plate with serrate processes from the anterior and posterior sides.

Alar expanse, 16–18 mm.

Type.—U.S.N.M. no. 58243.

Type locality.—Vero Beach, Fla.

Remarks.—Described from the ♂ type, one ♂ and seven ♀ paratypes all from the type locality. All were collected by John R. Malloch and bear April and May dates, 1941. Paratypes in the United States National Museum and the British Museum.

In pattern this species resembles *petalumensis* and is closely related to it as will be seen by a comparison of the genitalia. The male of *pereyra* lacks the sharply curved process of the harpe found in *petalumensis*, and the female of the latter species lacks the deeply excavated posterior edge of the terminal segment of *pereyra*.

Chionodes grandis, n. sp.

Figs. 4–4c

Labial palpus pale brownish gray, suffused, and irrorate with light fuscous. Antenna fuscous with indistinct, narrow, pale annulations. Head fuscous. Thorax and forewing dark purplish fuscous; on fold, at basal fourth, in the center of the cell and at the end of cell, ill-defined blackish-fuscous spots; at apical third on costa a small, indistinct, pale spot; cilia yellowish fuscous irrorate with blackish fuscous. Hind wing shining grayish fuscous; cilia yellowish fuscous basally, darker distally; hair pencil absent on underside of male. Legs shining fuscous; at bases of spurs and at ends of tarsal segments, small patches of ocherous-white. Abdomen shining fuscous with viola-

ceous cast; first three segments, dorsally, sordid yellow.

Male genitalia.—Symmetrical. Dorsal arm of harpe as long as tegumen and uncus combined, curved, slender, sharply pointed; ventral arm about one-third the length of the dorsal arm, filamentous. Aedeagus slender, about twice as long as dorsal arm of harpe; the slender stalk constituting about one-third the total length; apex enlarged and with two moderately sclerotized elongate plates directed away from apex. Gnathos a moderate sized hook.

Alar expanse, 25–28 mm.

Type.—U.S.N.M. no. 58242.

Type locality.—Silverton, Colo.

Remarks.—Described from the ♂ type and one ♂ paratype, both in the United States National Museum. The specimens were collected in July.

This is the largest species of the genus so far described. It resembles *C. permacta* (Braun) in wing shape and general appearance, but can be distinguished from that species by its larger size and the short, median, hooklike process of the uncus, which is about one-third the length of the median process of *permacta*.

ORNITHOLOGY.—*The races of the minivet* Pericrocotus peregrinus (*Linnaeus*) *in southeastern Asia.*[1]

H. G. Deignan, U. S. National Museum.

The extraordinary contradictions of Stuart Baker's successive treatments of this unfortunate minivet have been reviewed by Whistler and Kinnear (Journ. Bombay Nat. Hist. Soc. **36**: 343. 1933), who have at the same place set forth a sensible arrangement of the Indian races. It is now necessary that a similar revision be made of the forms occurring farther east.

Baker described *vividus* (see below) on characters of the male only, and gave its range as "Assam, Eastern Bengal, Manipur, Burma, Andamans, Siam, Cochin China." A series of 77 adults, chiefly from Siam, lying before me indicate that several distinct races, some of them with characters most evident in the females, have been hitherto embraced under this name. Owing to my complete lack of material from eastern Bengal, Assam, and most parts of Burma, a thorough study of "*vividus*" can not yet be presented; since, however, the type locality for the name lies on the frontiers of Siam, Siamese specimens are the ones of critical importance, and these are available in some abundance.

For the loan of their series of these birds, my thanks are due the authorities of the American Museum of Natural History and of the Academy of Natural Sciences of Philadelphia.

I now recognize the following southeastern Asiatic races:

1. Pericrocotus peregrinus thai, n. subsp.

Type.—Adult female, U.S.N.M. no. 336383, collected at Amphoe Chom Thong = Ban Luang (lat. 18°25′ N., long. 98°40′ E.), Chiang Mai Province, northwestern Siam, on February 9, 1937, by H. G. Deignan (original number 2315).

Diagnosis.—The adult female differs from those of *peregrinus* and *vividus* (in the restricted sense of this paper) by having the flanks and abdomen strongly washed with rich yellow or orange, rather than lightly washed with a less vivid yellow.

Baker's original description of "*vividus*" covers the characters of the adult male of *thai*. This reads: "Darker above than typical *peregrinus*; the throat generally rather blacker; the crimson [= flame-orange] breast brighter and extending lower on to the flanks and often on to the abdomen, remainder of the lower parts bright yellow with no, or practically no, white on the abdomen. Red on tail distinctly darker and more crimson [= orange]."

Range.—Burma (Thayetmyo District); Southern Shan States; Siam (north and east, south along the Mae Khong to the province of Ubon Ratcha Thani).

2. Pericrocotus peregrinus vividus Stuart Baker

Perecrocotus [*sic*] *peregrinus vividus* Stuart Baker, Bull. Brit. Orn. Club **40**: 114. Apr. 30, 1920

[1] Published by permission of the Secretary of the Smithsonian Institution. Received March 20, 1947.

("Attaran River," Amherst District, Tenasserim Division, Burma; type locality here restricted to Pabyouk, "a village near a group of isolated limestone rocks on the right bank of the Attaran R. about 22 miles in a direct line S.E. of Moulmein," *fide* Hume and Davison, Stray Feathers 6: 523. 1878).

Diagnosis.—The adult male is as described by Baker (see under *P. p. thai*), but in long series has the underparts perhaps a little less vivid, with a slight reduction of red and an increase of yellow, to approximate the race described next below.

The adult female differs from that of *thai* by having the flanks (and sometimes the abdomen) lightly washed with yellow.

In either sex the bill is slightly longer and heavier than that of *thai*, thus again showing approach to the following form.

Range.—Burma (Amherst District); Siam (southwest, reaching east to Bangkok).

Remarks.—The type locality for this race could scarcely have been more unwisely chosen! A line drawn from Moulmein in Tenasserim to Ban Rahaeng in western Siam represents the normal boundary between races of birds that vary between northwestern and southwestern Siam. The locality "Attaran River," as used by Davison, collector of the type specimen, means "between the old town of Attaran, distant about 34 miles S.E. of Moulmein and this latter" (*fide* Hume and Davison, *loc. cit. supra*, p. 522). The type thus came from some point on or just south of the dividing line and might well belong to a population exactly intermediate between *thai* and *vividus* as here understood. Inasmuch as a few poor specimens from Ban Rahaeng seem to belong to the more southern race, it is probable that those of Moulmein will also, but to improve the chances of the name *vividus*'s being properly employed for the population of southwestern Siam, I have now restricted the type locality to the most southern point on the Attaran River permissible by Hume and Davison's list of proveniences of the species (*loc. cit.*, p. 212). The type itself, as a male, is certain to be subspecifically unidentifiable, unless it possesses an exceptionally large bill, and the final disposition of the names *thai* and *vividus* must hang upon the characters of topotypical females, which are probably not yet represented in any museum. If, contrary to my expectation, *thai* must eventually be united with *vividus*, it is believed that the unnecessary name will not unduly burden a future reviser, since the population of Ban Luang, while clearly showing the characters of the race, come from a locality at no great distance from the Amherst District. In this event, *vividus*, as here understood, will require a new name.

3. Pericrocotus peregrinus sacerdos Riley

Pericrocotus peregrinus sacerdos Riley, Proc. Biol. Soc. Washington 53: 131. Nov. 8, 1940 (Sambor, Kompong Thom Province, Cambodia).

Diagnosis.—The adult male differs from those of *thai* and *vividus* by having the breast and rump rather more red, less orange, and the tips of the outer tail feathers more deeply colored.

The only adult female seen is inseparable from that of *vividus*.

Range.—Cambodia; Annam (south); Cochinchine?

Remarks.—Only five males of *sacerdos* have been available, but these seem to show that Riley's race may be recognized.

4. Pericrocotus peregrinus separatus, n. subsp.

Type.—Adult male, U.S.N.M. no. 173102, collected at Tanjong Badak, Mergui District, Tenasserim Division, Burma, on January 8, 1900, by W. L. Abbott.

Diagnosis.—The adult male differs from that of *vividus* (and so much the more from that of *thai*) by its decidedly longer and heavier bill, and from both by having the color of the underparts less vivid, the red being almost restricted to the upper breast, with the yellow accordingly of greater extent.

No adult female has been seen.

Range.—Burma (coasts and islands of the Mergui District); Siam (coasts and islands of the western side of the Peninsula from the Pak Chan Estuary to the Takua Pa Inlet).

Remarks.—This race, apparently confined to the mangroves of a limited part of the eastern shore of the Andaman Sea, is quite isolated from its relatives: *vividus* ranges southward in Tenasserim only to lat. 16°15′ N., and in southwestern Siam to lat. 11°40′ N., while *saturatus*

is restricted to the Greater Sundas, reaching Sumatra, but not the Malay Peninsula.

The large bill of *separatus* has already been commented upon by Robinson and Boden Kloss (Journ. Nat. Hist. Soc. Siam 5: 251. 1924).

It will be a matter of some interest to learn whether the female has the color characters of *vividus* or of *saturatus;* it may be expected to resemble the latter.

5. Pericrocotus peregrinus subsp.

Baron R. Snouckaert van Schauburg (Treubia 11: 311–312. 1930), with a single female before him, gave reasons for considering the birds of the Andaman Islands a distinct race. I have unfortunately but two adult males and one immature female from these islands. The males have the underparts as highly colored as the reddest examples of *thai* and the bill equal to that of *vividus* in size. Snouckaert van Schauburg's female had the underparts "wholly white, with a faint tinge of yellow on the flanks," thus evidently resembling the female of *saturatus* (and perhaps the still unknown female of *separatus*). Despite Whistler and Kinnear's dictum (Journ. Bombay Nat. Hist. Soc. 36: 344. 1933) that a series from the Andamans can not be separated from "*vividus,*" it appears that the Andaman population can not be combined with any one of the several forms now recognized, and, were a name available, I should gladly accept it. I am not willing, however, to set up a new subspecies without having seen more material, and especially females.

Snouckaert van Schauburg has suggested that "in case it might be found that a local subspecies could indeed be established, I should like proposing to name it after Mr. B. B. OSMASTON who collected my bird." His "type," an adult female taken at Port Blair, South Andaman Island, on April 3, 1905, was in his private collection; its present whereabouts is unknown.

6. Pericrocotus peregrinus saturatus Stuart Baker

Pericrocotus peregrinus saturatus Stuart Baker, Bull. Brit. Orn. Club 40: 115. Apr. 30, 1920 (West Java).

Diagnosis.—The adult male is inseparable from that of *vividus.*

The adult female has the flanks (and sometimes the abdomen) very lightly washed with pale yellow or cream and differs from that of *vividus* in the same degree as the female of *vividus* differs from that of *thai.*

Range.—Sumatra; Java; Bali.

Remarks.—Baker's setting up of the race *saturatus* throws an interesting light on his ornithological methods. In this particular revision he lists consecutively subspecies from (1) the Punjab, (2) Tenasserim, (3) the Malabar coast, (4) Sind, and (5) Java. No. 2 is discussed only in relation to no. 1, no. 3 only in relation to no. 2, and so on; in the end, the Javanese bird, no. 5, is distinguished, not from the geographically nearest form, but from the bird of Sind! Since the author considered the males of *vividus* and *saturatus* diagnostic (as is shown by his failure even to mention the females), it is certain that he could not have named the Javanese race at all, had he bothered to compare it with *vividus;* his "exceptionally dark birds above" has meaning only with reference to *pallidus!*

In any event, the population of Java, in which the male is like *vividus* and the female like *ceylonensis,* is a recognizable race, and Baker's name, however useless his type specimen, may properly be used for it.

I have seen no material from Sumatra and have simply followed other writers in including this island in the range of *saturatus.*